THE EFFECTS OF ENVIRONMENTAL CONDITIONS ON THE GROWTH, PHYSIOLOGY AND ULTRASTRUCTURE OF THE CYANOBACTERIUM ANACY 'IS NIDULANS

by

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To Pete

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DECLARATION

The work presented in this thesis was the result of original research carried out by myself under the supervision of Dr. J. H. Slater, none of which has, to my knowledge, been previously submitted for examination.

All sources of information contained in this thesis have been specifically acknowledged by means of references.

Linda M. Parrott.

SUMMARY

The effects of growth rate and light-and carbon dioxide-limitation on the growth, macromolecular composition, ultrastructure and carboxylase activity of the cyanobacterium Anacystis nidulans were determined using chemostat continuous-flow culture conditions over the dilution rate range, D=0.02 - 0.19 h^{-1}.

Under carbon dioxide-limiting conditions the culture showed the characteristic substrate-limited pattern of growth although under light-limiting conditions there was a rapid decrease in organism biomass with increasing dilution rate with the biomass concentration being proportional to the reciprocal of the dilution rate.

Growth rate in particular had a significant effect on the macromolecular content, in terms of DNA, RNA, protein, pigment and lipid, of A. nidulans whereas the nature of the growth-limiting substrate also had a significant effect on the carbohydrate content indicating that this was the main storage product in this organism.

Growth rate also had a significant effect on the RuBPCase activity of A. nidulans which tended to increase with increasing dilution rate under both limitations, except for high values obtained at very low dilution rates under carbon dioxide-limitation. The PEPCase activity tended to show an inverse correlation with that of RuBPCase activity whereas no pyruvate Case activity was obtained under any of the growth conditions examined.

Ultrastructure studies showed that this organism had basically the same typical prokaryotic fine structure irrespective of the dilution rate or substrate-limitation. The only exception was the presence of large densely stained inclusion bodies, which were probably polyphosphate granules, at D=0.02 h^{-1} under carbon dioxide-limitation. Some of the cellular components, such as the thylakoids, polyhedral bodies and ribosomes, did, however, alter quantitatively.

Competition studies of A. nidulans and the green alga Scenedesmus quadricauda showed that under carbon dioxide-limitation A. nidulans was the competitive dominant at all the dilution rates examined whereas under light-limitation A. nidulans was dominant at D=0.025 h^{-1} and S. quadricauda at D=0.035 and 0.045 h^{-1}.

PART 1. INTRODUCTION

The Cyanobacteria (so called by Stanier, Kunisawa, Mandel and Cohen-Bazire, 1971) or Cyanophyceae (according to Brock, 1973; Geitler, 1979) or blue-green algae occupy a unique position in the biological world. They, together with the bacteria are the only organisms with cells of the prokaryotic type (Stanier and van Niel, 1962; Echlin and Morris, 1965). They differ from the bacteria, however, in that they are aerobic photoautotrophs obtaining carbon and energy by photosynthetic mechanisms which are similar to those found in higher plants.

The Cyanobacteria include unicellular, colonial and filamentous forms, the most complex type of morphological structure obtained being the multiseriate branched filament (Fogg, Stewart, Fay and Walsby, 1973). These organisms show a greater extent of cellular differentiation and morphological elaboration than do bacteria with the most complex forms showing two major types of differentiated cell, the heterocyst, which is the cellular site of nitrogen fixation, and the akinete (or spore), a thick walled resting cell. Fritsch (1945) suggested a broad classification of these forms into five orders: Chroococcales, Chamaesiphonales, Pleurocapsales, Nostocales and Stigonematales. However, more recently Rippka, Deruelles, Waterbury, Herdman and Stanier (1979) re-classified these organisms into five large sub-groups on a generic as well as a morphological, biochemical and physiological basis.

The Cyanobacteria are an ancient group of organisms which probably arose from a photosynthetic bacterium, possibly Chloroflexis (Fogg et al., 1973). Fossil evidence indicates that unicellular

forms had developed by the Early Precambrian and that non-heterocystous and heterocystous filamentous forms were abundant by the Middle Precambrian era (Schopf, 1970). It is virtually certain that cyanobacteria were the first oxygen-producing photosynthetic organisms and were probably responsible for the transition from an anaerobic to an aerobic atmosphere (Schopf, 1970). The preference of living cyanobacteria for minimal oxygen concentrations and their great resistance to ultra-violet radiation provides further evidence that this group appeared early on in biochemical evolution (Fogg et al., 1973). It is now believed that the ancestors of chloroplasts might have been these photosynthetic prokaryotes which became endosymbionts of primitive eukaryotes and subsequently lost their genetic independence (Stanier and Cohen-Bazire, 1977).

Today these organisms show a world wide distribution which is presumably related to both the antiquity of the group and the high degree of resistance which most of its members show towards adverse conditions, such as extremes of temperature, desiccation and high salt concentrations (Fogg et al., 1973). On one hand they predominate in the flora of arid deserts and are abundant on exposed rocks and buildings while on the other different species show massive growth in temperate freshwater lakes, especially when polluted. Also, they are the dominant plant life in frigid lakes of the Antartic as well as growing in hot springs with an upper temperature limit of 72 - 73°C (Brock, 1973). These organisms seem to be widely distributed in practically all habitats which will support life being rivalled only by the bacteria in the range of habitats they can occupy. They tend to prefer neutral or alkaline conditions

(pH 7.5 - 9.0) although, for example, Chroococcus turgidus is common in Sphagnum bogs at approximately pH 4.0. Cyanobacteria are widely distributed in terrestrial habitats occurring predominately on the surface of soils, stones, rocks and trees and are abundant in cultivated soils, especially in rice paddy fields. They play a major role as primary colonisers which help in the establishment of other members of the soil flora and in the accumulation of humus, for example, seven species were found in the pioneer flora which had established itself on the volcanic Island of Surtsey off Iceland only five years after its formation (Fogg et al., 1973). They may also be abundant in freshwater habitats, in both static and running waters, as planktonic, epilithic and epiphytic forms (Fogg et al., 1973; Whitton, 1973a) and in marine ecosystems, being particularly abundant in estuarine and intertidal areas, as well as being found on sandy and rocky shores, in salt marshes and marine muds and in the open ocean as benthic and planktonic forms (Fogg, 1973; Fogg et al., 1973).

Cyanobacteria may also exist in close association with a wide range of other organisms and in many cases true mutualism seems to occur with both organisms benefitting from the association at least under certain conditions (Whitton, 1973b). Symbiotic associations may occur with, for example, fungi as in lichens, bryophytes, such as species of Anthoceros, Blasia and Cavicularia, pteridophytes, such as the water fern Azolla, cycads (gymnosperms), Gunnera sp. (angiosperms) and various sponges, such as Ircinia variabilis (metazoa) (Holm-Hansen, 1968; Fogg et al., 1973). On

the other hand, various other organisms, such as species of protozoa, fungi, bacteria and viruses (cyanophages) may be antagonistic towards cyanophyta and cause cell lysis (Fogg et al., 1973; Redhead and Wright, 1978; Safferman, 1973; Whitton, 1973b). Also, many bloom-producing cyanobacteria, such as Microcystis sp., Anabaena sp. and Aphanizomenon sp., can themselves produce toxic factors that can result in the mortality of other cyanobacterial species, eukaryotic algae, fish, wildfowl and domestic animals (Fogg et al., 1973). Where these substances are effective against other algae, which might compete for light or nutrients, or predatory organisms their survival value is clear but their toxicity to other organisms which present no obvious threat is perhaps fortuitous. In contrast, other extracellular substances produced by cyanobacteria, such as nitrogenous compounds, may be utilised by other organisms, such as bacteria, algae, fungi and even higher plants, such as rice (Whitton, 1973b).

Cyanobacteria are the only group of oxygen-evolving photosynthetic organisms which are capable of nitrogen fixation (Brock, 1973), biological nitrogen fixation being defined as the enzymic conversion of dinitrogen from the atmosphere to ammonia by the enzyme-complex nitrogenase (Stewart, 1973). The capacity to fix nitrogen appears to be rare in unicellular forms but has been found under aerobic conditions in Gloeocapsa sp. (Wyatt and Silvey, 1969) and Aphanothece sp. (Stanier and Cohen-Bazire, 1977). Various non-heterocystous filamentous forms may also fix nitrogen but only under microaerophilic conditions (Stewart and Lex, 1970; Rippka and Waterbury, 1977). The most important contributors of

combined nitrogen in aerobic environments are the heterocystous cyanobacteria (Fogg et al., 1973). Heterocysts are characteristic of the Nostocales (except Oscillatoriaceae) and Stigonemateles (Rippka et al., 1979) and are the sites of nitrogen fixation (Stewart, Haystead and Pearson, 1969; Neilson, Rippka and Kunisawa, 1971). They were found to contain nitrogenase activity and PSI activity which was capable of generating ATP so providing energy for nitrogen fixation (Fay, 1969). However, they lacked the pigments necessary for PSII activity and did not evolve oxygen (Tel-Or and Stewart, 1977) so protecting the nitrogenase enzyme from inactivation by oxygen.

There seems to be increasing evidence that nitrogen-fixing cyanobacteria could make a major contribution to the fertility of certain specialised tropical habitats, especially paddy fields. Also, free-living cyanobacteria and lichens may be major contributers of combined nitrogen in the Arctic and Antartic where turnover rates are low. In addition these organisms may be important as nitrogen fixers in arid desert soils, temperate soils, tundra and aquatic ecosystems, such as freshwater lakes and streams, hot springs and the marine environment (Fogg et al., 1973; Stewart, 1973).

Cyanobacteria are also important in increasing the fertility of terrestrial and aquatic environments, in colonising newly exposed land surfaces, in reducing soil erosion and possibly producing growth factors for higher plants (Holm-Hansen, 1968; Fogg et al., 1973). These organisms could possibly even be used

as a food source or food supplement, such as a source of vitamin B_{12} (Fogg et al., 1973). On the other hand, these organisms may become a nuisance in freshwater habitats where dense blooms of certain species can occur, especially in polluted waters. These organisms can grow in such profusion that they result in serious losses of economic and aesthetic resources (Holm-Hansen, 1968).

The Cyanobacteria, which were probably predominant among the aboriginal organisms on the Earth, are still, therefore, quantitatively and qualitatively important in the biosphere and the study of these remarkable organisms is not only of particular intrinsic interest for biology but is also of economic importance (Fogg et al., 1973).

1.1. METABOLIC PATHWAYS IN CYANOBACTERIA

1.1.1. Light reactions of photosynthesis

Cyanobacteria, like eukaryotic algae and higher plants, normally use water as the ultimate hydrogen or electron donor in photosynthesis with the elimination of molecular oxygen as a means of disposal of the oxidising fragment (Fogg et al., 1973). However, in common with certain algae, these organisms are also capable of utilising other hydrogen donors, such as molecular hydrogen, H_2S and Na_2S, in a process of photoreduction which resembles bacterial photosynthesis (Fogg et al., 1973; Cohen, Jørgensen, Padan and Shilo, 1975; Belkin and Padan, 1978; Oren and Padan, 1978).

The utilisation of light by cyanobacteria involves two distinct photosystems (known as PSI and PSII) linked in series,

with ATP being produced both by non-cyclic and cyclic photophosphorylation and the reductant for carbon dioxide fixation being generated as NADPH. The PSII reaction centre, chl a_{680}, collects the light which is absorbed mainly by the phycobiliprotein accessory pigments and uses this light energy to excite the electrons generated on the photolysis of water. These electrons are transferred via a series of electron carriers, among which plastoquinone, plastocyanin and cytochrome c_{554} have been identified and others, such as α-tocopherolquinone and vitamin K, implicated, to the PSI reaction centre P_{700}, generating ATP in the process. The electrons are excited again, mainly by light energy absorbed by chlorophyll a, and raised to the level of iron-sulphur proteins before returning either to generate ATP by cyclic photophosphorylation or to produce reductant in the form of NADPH (Fogg et al., 1973; Krogmann, 1973; Stewart, 1978).

The phycobiliproteins are the principal photosynthetic accessory pigments in cyanobacteria, their characteristic absorption spectra complementing those of the other photosynthetic pigments chlorophyll a and carotenoids. Although light energy absorbed by these pigments appears to be primarily transferred to PSII, it can also be conveyed into PSI (Jones and Myers, 1964; Wang, Stevens and Myers, 1977). Chemically the phycobiliproteins consist of non-cyclic tetrapyrrolic bile pigments covalently bound to a protein. Phycocyanin and allophycocyanin are believed to be universally present in all cyanobacteria with phycoerythrin present in some species (Chapman, 1973). These pigments were

found to transfer absorbed light energy to other components of the photochemical system very efficiently (Emerson and Lewis, 1942; Duysens, 1951) indicating that the interacting pigment molecules were in close association (Arnold and Oppenheimer, 1950). The phycobiliproteins have since been found as aggregates in the form of phycobilisomes on the outer surfaces of thylakoid membranes (section 1.3.7.6.) and the pathway of energy transfer has been indicated to be from phycoerythrin to phycocyanin to allophycocyanin to allophycocyanin B to chlorophyll a (Glazer, 1977). However, Csatorday, Kleinen Hammans and Goedheer (1978) showed that direct energy transfer between phycocyanin and chlorophyll a, by-passing the excitation energy transfer chain through allophycocyanin and allophycocyanin B, might exist in the phycobilisome of A. nidulans.

The other major group of pigments present in these organisms are the carotenoids, of which there are two basic categories: the carotenes which are hydrocarbons and the xanthophylls which are oxygen-containing derivatives (Nakayama, 1962; Fogg et al., 1973; Stanier and Cohen-Bazire, 1977). β-carotene appears to be universal and is often the principal carotenoid and only carotene present although others, such as flavacene, may occur in some species and this is usually accompanied by either or both of the xanthophylls zeaxanthin and echinenone, plus other group specific xanthophylls, such as oscilloxanthin. According to Emerson and Lewis (1942) light absorbed by carotenoid pigments seemed to be mainly unavailable for photosynthesis. It was concluded that light absorbed by carotenoids might be

active in photosynthesis but with a considerably lower efficiency than that of chlorophyll and phycocyanin or that light absorbed by one or a few carotenoid components was utilised with a high efficiency while light absorbed by most of the components was lost for photosynthesis. Goedheer (1969) has since found that energy transfer from β-carotene to chlorophyll occurred with a very high efficiency but only in PSI, which according to Ogawa, Vernon and Mollenhauer (1969) contained a high ratio of β-carotene to total xanthophylls, and that xanthophylls did not transfer energy to the chlorophyll of either photosystem. Apart from a photosynthetic function, carotenoids have also been implicated in providing photoprotection for chlorophyll a in the photosynthetic membranes (Emerson and Lewis, 1942; Nakayama, 1962) and in playing an important role in membrane organisation where they may be involved in structural and possibly energetical coupling between the phycobilisomes and the thylakoid membrane (Szalontai and Csatorday, 1979).

1.1.2. Carbon dioxide fixation

1.1.2.1. Calvin cycle

The Calvin cycle (reductive pentose phosphate pathway) has been confirmed as the principal route of net carbon dioxide assimilation in cyanobacteria (Pelroy and Bassham, 1972; Fogg et al., 1973; Smith, 1973; Ihlenfeldt and Gibson, 1975a). In this cycle (figure 1.1.) carbon dioxide combines with a 5-carbon acceptor substance, ribulose bisphosphate (RuBP), which splits

Figure 1.1. The Calvin cycle (reductive pentose phosphate pathway).

(Redrawn from Fogg et al., 1973)

RuBP : ribulose 1,5-bisphosphate

3PGA : 3-phosphoglyceric acid

BPGA : 1,3-bisphosphoglyceric acid

GAP : glyceraldehyde-3-phosphate

DHAP : dihydoxyacetone phosphate

FBP : fructose 1,6-bisphosphate

F6P : fructose 6-phosphate

G6P : glucose 6-phosphate

E4P : erythrose 4-phosphate

SHBP : sedoheptulose 1,7-bisphosphate

SH7P : sedoheptulose 7-phosphate

Xu5P : xylulose 5-phosphate

R5P : ribose 5-phosphate

Ru5P : ribulose 5-phosphate

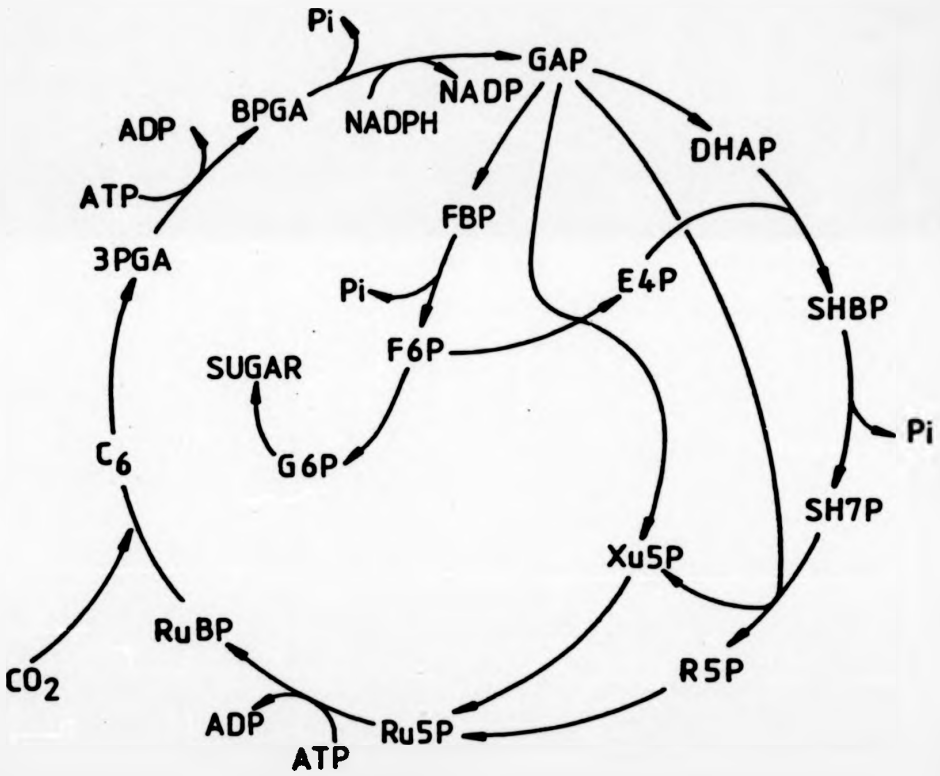
C_6 : C_6 compound

ATP : adenosine triphosphate

ADP : adenosine diphosphate

Pi : inorganic phosphate

NADPH/NADP : reduced and oxidized forms of nicotinamide adenine dinucleotide phosphate


Pi
GAP
NADP
NADPH
BPGA
ADP
ATP
DHAP
FBP
3PGA
Pi
E4P
SHBP
F6P
SUGAR
Pi
G6P
C6
SH7P
Xu5P
RuBP
CO2
R5P
ADP
Ru5P
ATP

to yield two molecules of a 3-carbon substance, 3-phosphoglyceric acid (PGA). This is then reduced to a triose sugar phosphate from which, by a series of sugar phosphate interconversions, the acceptor for carbon dioxide (that is, RuBP) is regenerated with a net gain of a triose which is characteristically converted to glycogen. This cycle could produce one molecule of glucose for every six carbon dioxide molecules fed into it but, in fact, fixed carbon is removed in a variety of intermediate forms for the synthesis of cell products. The energy required to drive these reactions is supplied by ATP and NADPH from the photochemical reactions (section 1.1.1.).

The carboxylating enzyme of the Calvin cycle is ribulose 1,5-bisphosphate carboxylase (RuBPCase) which, according to Glover and Morris (1979), is present in all autotrophic organisms. RuBPCase from all the eukaryotic sources so far examined had a high molecular weight (500,000 - 550,000 daltons) and contained two classes of subunits with molecular weights of approximately 55,000 (large, L) and 15,000 (small, S) arranged in a L_8S_8 quaternary structure (McFadden and Purohit, 1978). A similar size and subunit composition has been found for the RuBPCases of the cyanobacteria Aphanocapsa 6308 (Codd and Stewart, 1977a), Microcystis aeruginosa (Stewart, Auchterlonie and Codd, 1977), Plectonema boryanum and Anabaena variabilis (Takabe, Nishimura and Akazawa, 1976) and Anabaena cylindrica (Takabe, 1977). However, small subunits have not been revealed in the RuBPCases of other cyanobacteria, such as Agmenellum quadruplicatum

(Tabita, Stevens and Gibson, 1976) and Aphanothece halophytica (Codd, Cook and Stewart, 1979). An even greater diversity in size and subunit structure seems to be a characteristic of the RuBPCase of bacteria, with both subunits present in some organisms, such as Rhodopseudomonas spheroides (Gibson and Tabita, 1977) and Thiobacillus neapolitanus (Snead and Shively, 1978) but large subunits only present in others, for example, in a dimeric state, Rhodospirillum rubrum (Tabita and McFadden, 1974), a hexameric state, Chlorobium thiosulphatophilum (Tabita, McFadden and Pfenning, 1974) and an octameric state, Thiobacillus intermedius (Purohit, McFadden and Cohen, 1976a). A scheme for the molecular evolution of RuBPCase has been proposed by McFadden and Tabita (1974) which envisages the evolution from an ancestral L enzyme to L_2, L_4, L_8 and eventually L_8S_8 types.

The large subunits of RuBPCase seem to be more or less homologous in primary structure (amino acid composition) from R. rubrum through to the higher plants (McFadden and Purohit, 1978). It has been suggested that the evolutionary conservation of this structure is due to the fact that the large subunits are primarily responsible for enzyme activation and catalysis (Takabe et al., 1976; Codd and Stewart, 1977a; Kobayashi, Takabe, Nishimura and Akazawa, 1979). In contrast, the small subunits vary even among different genera of higher plants and their precise function is unknown. It has been suggested that they may be involved in sustaining the conformation of the enzyme molecule which is necessary for effective activation (Kobayashi et al., 1979) or may serve as membrane attachment sites to anchor the catalytically

functional large subunits (McFadden and Purohit, 1978) or may be closely associated with the regulatory mechanism of the enzyme reaction related to the Mg^{2+} effect (Takabe et al., 1976).

According to McFadden (1973), RuBPCase is one of the slowest catalysts in nature and the natural occurence of unusually large amounts of this enzyme may compensate for the low turnover numbers observed, especially as the enzyme is probably not saturated by substrate(s) in vivo (McFadden and Tabita, 1974). RuBPCase activity in tissue extracts, assayed with saturating substrate concentrations has indeed generally been found to be only just enough, and quite often inadequate, to account for the rates of photosynthesis found in intact cells (Hatch and Slack, 1970), although McFadden (1973) has indicated that the kinetic properties of RuBPCase may be altered when the enzyme is removed from its natural environment.

In higher plants and green algae RuBPCase is located in the chloroplasts as are other enzymes which function in the Calvin cycle. In prokaryotes RuBPCase is frequently located in polyhedral bodies known as carboxysomes (section 1.3.7.5.) although in certain prokaryotic autotrophs, including photolithotrophic bacteria and numerous chemolithotrophic bacteria, these bodies were not evident (McFadden and Purohit, 1978). Also, RuBPCase was found to be absent from the heterocysts of A. cylindrica which was consistent with the view that heterocysts cannot photoassimilate carbon dioxide (Codd and Stewart, 1977b).

In general this enzyme seemed to have an absolute requirement

for magnesium ions for activity (McFadden and Purohit, 1978) and the large molecular weight RuBPCases were found to be sensitive to inhibition by 6-phospho-D-gluconate (6-PG), a component of the oxidative pentose phosphate pathway (Tabita and McFadden, 1972; McFadden, 1973). A number of other compounds which contained phosphate groups, especially the adenine nucleotides, were also found to cause significant inhibition of RuBPCase activity in Thiobacillus novellus (McCarthy and Charles, 1973; 1975).

A number of other factors, including environmental conditions, have been found to affect the activity of this enzyme. For example, oxygen has been found to inhibit the activity of RuBPCases from higher plants to anaerobic bacteria (McFadden and Tabita, 1974). Oxygen acted as a competitive inhibitor with respect to carbon dioxide suggesting that the enzyme could also act as an oxygenase. According to McFadden and Purohit (1978) the oxygenase activity of RuBPCase accounts for, or makes a major contribution to, photorespiration which is defined as a light dependent uptake of oxygen and release of carbon dioxide during photosynthesis. This process therefore opposes photosynthesis due to a loss of carbon dioxide so lowering the growth rate of the organism. In the RuBP oxygenase reaction RuBP is split into PGA (the normal product of the carboxylase reaction) and phosphoglycolate. The phosphoglycolate may be metabolised by the glycolate pathway as normally found in higher plants, such as soybean (Bowes, Ogren and Hageman, 1971), or via tartronic semialdehyde as shown in A. cylindrica (Codd and Stewart, 1973).

Both of these pathways were indicated in Anacystis nidulans (Halldal and Holmen, 1979). Despite these reports and others, such as those of Codd and Stewart (1977a) for Aphanocapsa 6308 and McFadden, Lord, Rowe and Dilks (1975) for Euglena gracilis, suggesting an oxygenase activity for the RuBPCases of algae, Lloyd, Canvin and Culver (1977) reported that under normal conditions of growth glycolate formation and photorespiration seemed to be absent due to a lack of effect of oxygen on apparent photosynthesis in a number of algae including Anabaena flos-aquae and A. nidulans. A similar conclusion was reported by Bidwell (1977) showing that Chlorella pyrenoidosa, Chlamydomonas reinhardii and A. flos-aquae lacked conventional photorespiration but might carry on a dark type respiration in the light which was oxygen insensitive but affected by carbon dioxide.

The carboxylase activity of this enzyme has been found in certain cases to be affected by the concentration of one of its substrates, carbon dioxide. RuBPCase activity was shown to increase due to carbon dioxide-limitation in several organisms, for example, T. neapolitanus (Kuenen and Veldkamp, 1973; Beudeker, Cannon, Kuenen and Shively, 1980) and A. nidulans (Karagouni, 1979; Karagouni and Slater, 1979) although the activity of RuBPCase from Thiomicrospira pelophila did not change due to this limitation (Kuenen and Veldkamp, 1973). Slater and Morris (1973) reported that there was generally a positive correlation between the rate of photosynthetic carbon dioxide fixation and RuBPCase activity in autotrophically and photoheterotrophically grown

R. rubrum and a similar correlation was shown for a number of phytoplankton species (Glover and Morris, 1979). In contrast, Beudeker et al., (1980) found an inverse correlation between RuBPCase activity and the carbon dioxide fixation capacity by whole cells of T. neapolitanus and Karagouni and Slater (1979) found that in neither light-nor carbon dioxide-limited chemostat cultures of A. nidulans did the changing pattern of carbon dioxide fixation by intact organisms correlate with the observed RuBPCase activities. Karagouni and Slater (1979) also reported that the RuBPCase activity in vitro accounted for only 10% of the in vivo rate of carbon dioxide assimilation required for the growth of A. nidulans at high dilution rates so reflecting the problems of correlating enzyme activity with the behaviour of intact organisms.

Growth rate may affect RuBPCase activity in certain organisms. In thiosulphate-limited cultures of T. neapolitanus a slight decrease in activity was measured with increasing growth rate although in T. pelophila RuBPCase activity was almost independent of growth rate over a wide range (Kuenen and Veldkamp, 1973). Slater (1975) reported a four-fold increase in RuBPCase activity on a per cell basis for light-limited chemostat cultures of A. nidulans over the growth rate range 0.02 - 0.10 h^{-1} although in terms of total protein the activity remained constant at these growth rates. In contrast Karagouni (1979) and Karagouni and Slater (1979) reported that under the same conditions of light-limitation over the growth rate range 0.02 - 0.19 h^{-1} the

RuBPCase activity remained constant both in terms of total protein and unit cell number. These workers also reported a 15-fold increase of RuBPCase activity on a protein and unit cell basis in carbon dioxide-limited cultures of A. nidulans with decreasing growth rate. It was concluded that the alterations in the RuBPCase activity was probably in response to changes in the external carbon dioxide concentration rather than different growth rates since under light-limited conditions with carbon dioxide in excess at all dilution rates examined there was no change in the specific activity of the RuBPCase. The results also suggested that this enzyme was under transcriptional control in this organism in contrast to the suggestion of Carr (1973a) that cyanobacteria cannot in general control their enzyme activities at the transcriptional level and as a consequence are obligate autotrophs.

Temperature was also found to affect the RuBPCase activity of A. nidulans (Karagouni, 1979). Low temperature (25°C rather than 40°C) was found to cause an increase in the specific activity of the RuBPCase under both light-and carbon dioxide-limitations. In addition the specific activity under light-limitation was found to increase with increasing growth rate rather than remaining constant as at 40°C. The rate of carbon dioxide assimilation was found to be depressed at low growth temperatures so it was concluded that the increased RuBPCase levels at temperatures lower than optimal could presumably scavenge the available carbon dioxide in a similar manner to that shown at low carbon dioxide

concentrations when a high specific activity of RuBPCase was also found.

Light intensity may affect RuBPCase activity although reports are contradictory. Lascelles (1960) showed that high light intensities reduced RuBPCase levels in R. spheroides whereas the specific activity of RuBPCase in acetate grown R. rubrum at high light intensity (835 ft-c) was 2.5-fold higher than after growth at low light intensity (140 ft-c) (Porter and Merrett, 1972). The age of a culture may also affect the RuBPCase activity which was shown to increase in T. intermedius (Purohit, McFadden and Shaykh, 1976b) and Paracoccus denitrificans (Shively, Saluja and McFadden, 1978) and remain stable in Thiobacillus denitrificans (McFadden and Denend, 1972) during the exponential growth phase and then decrease slightly during stationary phase. Similar results were obtained for the cyanobacteria Anabaena CA and A. quadruplicatum with the RuBPCase steadily increasing during exponential growth of these organisms (Tabita, Caruso and Whitman, 1978).

Generally when bacteria are grown autotrophically they contain RuBPCase in large amounts with a high specific activity but when grown heterotrophically this activity is usually drastically reduced as shown for T. novellus (Aleem and Huang, 1965), T. intermedius (Purohit et al., 1976b), R. spheroides (Lascelles, 1960) and R. rubrum (Anderson and Fuller, 1967; Slater and Morris, 1973). McCarthy and Charles (1974) showed similar results for T. novellus and although on conversion to a

heterotrophic mode of growth a relatively slow decline of RuBPCase was shown, there was no enzyme detectable after 24 hours. However, under some conditions of heterotrophic culture, high rates of RuBPCase were sustained, for example, in Hydrogenomonas facilis and Hydrogenomonas eutropha when grown on fructose when the aeration was low, $FeCl_3$ added and the cells harvested prior to or at the mid-exponential phase of growth (Kuehn and McFadden, 1968) and in R. rubrum when grown on butyrate in the light (Tabita and McFadden, 1974). On the other hand, under normal heterotrophic growth conditions the RuBPCase activity in cell-free extracts of the obligate chemolithotrophs, T. pelophila, Thiobacillus thioparus and T. neapolitanus, was independent of the presence of exogenous organic compounds,such as acetate and succinate (Kuenen and Veldkamp, 1973). No change in the enzyme could be demonstrated either in thiosulphate-or carbon dioxide-limited cultures. These organisms were shown to lack an operative tricarboxylic acid cycle and glyoxylic acid cycle as do cyanobacteria (section 1.1.3.) (Smith, London and Stanier, 1967) and, in general, the RuBPCase activity of the latter also does not seem to be significantly affected by the presence of exogenous organic compounds. Pearce and Carr (1967a) reported preliminary results which indicated that RuBPCase activity in A. nidulans and A. variabilis was only slightly altered after growth in the presence of sodium acetate and Harrison and Carr (unpublished observations quoted in Carr, 1973b) found that neither glucose

nor pyruvate repressed RuBPCase activity in A. variabilis. Harrison and Carr (unpublished observations quoted in Carr, 1973a) also found no alteration of RuBPCase activity in cyanobacteria after the inclusion of sugars in the phototrophic growth medium and reported that RuBPCase activity in dark heterotrophic cultures of Chlorogloea fritschii was still the same as in phototrophic cultures after 8 weeks of growth. However, after a period of one year's maintenance in the dark the activity had dropped to approximately 7% of the phototrophic culture. This observed decline in enzyme activity could possibly be accounted for due to the fact that the growth rate of the organism under dark heterotrophic conditions was at least 10-fold less than in the light. Joset-Espardellier, Astier, Evans and Carr (1978) working on Aphanocapsa 6714 and C. fritschii also showed little variation in enzyme level under photoautotrophic, photoheterotrophic and heterotrophic growth conditions. In both organisms the RuBPCase activity decreased slightly under photo-heterotrophic growth conditions but actually increased under heterotrophic growth conditions in C. fritschii although remaining constant in Aphanocapsa 6714 as under photoautotrophic growth conditions. In contrast to these results, Carr, Hood and Pearce (1969) have presented evidence suggesting that the presence of acetate in the growth medium of A. variabilis caused a reduction in the RuBPCase activity compared with the autotrophically grown control organisms.

1.1.2.2. β-carboxylations

β-carboxylations catalyse the conversion of C_3 to C_4 compounds and occur in all living organisms (Glover and Morris, 1979) being important in both autotrophic and heterotrophic growth of higher algae and cyanobacteria (Benedict, 1978).

In the so-called C_4 plants, such as sugar cane, a β-carboxylation, catalysed by phosphoenol pyruvate carboxylase (PEPCase), plays a primary role in photosynthetic carbon assimilation with bicarbonate ions being fixed to form a C_4 compound, oxaloacetate, which is in equilibrium with malate and aspartate (Hatch and Slack, 1970). It has been postulated that a decarboxylation reaction occurs liberating carbon dioxide for refixation by RuBPCase and pyruvate which is then phosphorylated to regenerate the carbon dioxide acceptor phosphoenol pyruvate (PEP). Consequently, RuBPCase and the Calvin cycle are still required for net carbon dioxide fixation.

Ihlenfeldt and Gibson (1975a) suggested that this dicarboxylic acid pathway might play an important role in the metabolism of cyanobacteria as organisms of this group are found in environments of high light intensities and low carbon dioxide tension. However, the results obtained substantiated the views of Jansz and Maclean (1973) that the Calvin cycle was the major route of carbon dioxide fixation with the carboxylation of PEP being a minor pathway of carbon dioxide incorporation. Ihlenfeldt and Gibson (1975a) concluded that the usually much

lower K_m measured for PEP carboxylation could make this reaction more significant quantitatively at low carbon dioxide tensions but that it seemed unlikely that a C_4 fixation pathway such as that found in certain higher plants could ever dominate in A. nidulans, especially as Jansz and Maclean (1973) were unable to detect any transcarboxylase activity.

In contrast to these results Colman, Cheng and Ingle (1976) found relatively high levels of activity of PEPCase in A. flos-aquae, A. nidulans and Oscillatoria sp. in preparations obtained by lysis of spheroplasts by lysozyme. The activity of PEPCase was found to be 1.5 to 5-fold higher at 20°C than that of RuBPCase in contrast to the activities found for C. pyrenoidosa, a known C_3 alga in which RuBPCase was 25-fold more active. It was suggested that the low levels of PEPCase activity previously reported (Jansz and Maclean, 1973) were due to the loss of enzyme activity caused both by the severity of the method of breaking the cells and the use of Tris-HCl buffer which was found to accelerate the instability of this enzyme in comparison with phosphate buffer. It was concluded that due to the high levels of PEPCase activity and the possession of many of the characteristics common to C_4 plants, such as a high optimum temperature for photosynthesis, a low rate of photorespiration and low levels of carbonic anhydrase compared with C_3 plants, that the cyanobacteria studied had the ability to fix carbon dioxide by a C_4 pathway. Further evidence for a C_4 pathway in cyanobacteria was provided by Colman and Coleman (1978) who

found that malonate (an inhibitor of PEPCase) had a marked inhibitory effect on photosynthetic carbon dioxide fixation in Coccochloris peniocystis, A. flos-aquae, A. nidulans and Oscillatoria sp. This conclusion was obviously inconsistent with reports that the first detectable product of carbon dioxide fixation in cyanobacteria was PGA (Pelroy and Bassham, 1972; Ihlenfeldt and Gibson, 1975a) which would indicate the operation of the Calvin cycle. However, according to Colman and Coleman (1978) these studies were not an unequivocal demonstration of the C_3 pathway in cyanobacteria as the organisms were grown on elevated levels of carbon dioxide which would be expected to repress the activity of carbonic anhydrase and repress the bicarbonate transport systems. Under these conditions high carbon dioxide grown cells would photosynthetically utilise only carbon dioxide diffusing into the cell and since the substrate of PEPCase is bicarbonate no incorporation into C_4 acids would occur.

The data of Weathers and Allen (1978) also suggested an operative C_4 pathway in Aphanocapsa 6308 as well as confirming the importance of the C_3 pathway in carbon dicxide fixation. The C_4 pathway did not seem to be operative, however, for purposes related to carbohydrate synthesis but rather as a compensation for the incomplete tricarboxylic acid cycle (section 1.1.3.) in cyanobacteria (a suggestion also put forward by Ihlenfeldt and Gibson (1975a)). It seemed that in this organism C_4 fixation

into malate and aspartate played a major role by offering additional fixation sites to compensate for demands for aspartate production, especially in stationary phase cells.

PEPCase has also been found in a number of bacteria, including Escherichia coli in which catalytic quantities of acetyl-CoA were required for activity (Cooper and Kornberg, 1967) whereas acetyl-CoA was found to have no effect on the activity of PEPCase from A. cylindrica (Codd and Stewart, 1973).

Glover and Morris (1979) working on phytoplankton cultures did not find a positive correlation of PEPCase activity with photosynthesis in contrast to the RuBPCase activity. It seemed that PEPCase was quantitatively important only when the rate of photosynthesis was low as the RuBPCase:PEPCase ratios were high in exponential growth phase cultures when photosynthesis was maximal and low in stationary phase cultures when photosynthesis was reduced. A similar observation was reported by Benedict (1978) who stated that under poor growth conditions in poorly aerated systems β-carboxylation was the major means of carbon dioxide fixation in algae. For example, in low light intensities which did not allow net photosynthesis in Chlorella. C_4 dicarboxylic acids and citrulline were the primary products of carbon dioxide fixation.

The effect of heterotrophic growth on the activity of PEPCase has been determined in bacteria. In T. novellus PEPCase activity was virtually unchanged under autotrophic or heterotrophic growth conditions (Aleem and Huang, 1965) whereas in

another study on the same organism both PEPCase and pyruvate Case (another β-carboxylation enzyme) activity was found to increase 7-fold and 6-fold respectively on conversion to a heterotrophic mode of growth (McCarthy and Charles, 1974).

Pyruvate Case has also been found to be active in other bacterial species, including Bacillus coagulans (Cazzulo, Sundaram and Kornberg, 1969) and R. spheroides (Payne and Morris, 1969) in which it catalysed the carboxylation of pyruvate to yield oxaloacetate. This activity was found to be acetyl-CoA dependent. However, Codd and Stewart (1973) were unable to detect pyruvate Case activity in A. cylindrica even when acetyl-CoA was added.

In other bacterial species, such as E. coli (Cooper and Kornberg, 1967) and C. thiosulphatophilum. Chromatium sp. and R. rubrum (Buchanan and Evans, 1966) pyruvate was found to be converted to PEP at the expense of ATP by means of the enzyme PEP synthetase. The PEP formed could then be carboxylated to oxaloacetate in a reaction catalysed by PEPCase.

If cyanobacteria do possess a C_4 dicarboxylic acid pathway then the presence of PEP synthetase would be expected in these organisms to regenerate the carbon dioxide acceptor PEP from pyruvate. However, indirect evidence from the patterns of labelling in cells which assimilated ^{14}C pyruvate indicated that PEP synthetase was absent in these organisms (Smith et al., 1967) with radioactivity being restricted essentially to valine, alanine, leucine and the amino acids of the glutamic acid family. Aspartate, threonine, serine and glycine did not

incorporate radioactivity from pyruvate showing that under these growth conditions at least the organisms were unable to use pyruvate as a precursor of oxaloacetate. It was presumed that PEP was the immediate precursor of oxaloacetate under these conditions so substantiating the view of Weathers and Allen (1978) that PEPCase was present to compensate for the incomplete tricarboxylic acid cycle. This conclusion was, however, made from indirect evidence as no attempt was made to demonstrate the presence of pyruvate synthetase in cell-free extracts. Also, it was possible that under the growth conditions used, with an elevated carbon dioxide level, this enzyme would not have been active anyway. According to Colman and Coleman (1978) under these conditions the C_4 pathway would not be expected to operate. So this cannot be taken as definite evidence against the possible operation of this pathway in cyanobacteria.

1.1.3. Respiration

Carbohydrate dissimilation in cyanobacteria is primarily via the oxidative pentose phosphate pathway (Cheung and Gibbs, 1966; Pearce and Carr, 1969; Pelroy and Bassham, 1972; Pelroy, Rippka and Stanier, 1972; Lex and Carr, 1974), with glycolysis, the tricarboxylic acid (TCA) cycle and fermentation being unimportant in these organisms as sources of reductant (Stewart, 1978). The dissimilatory pathway seems to be a respiratory one as in the dark glucose metabolism was almost completely prevented by anaerobiosis (Pelroy et al., 1972) and an electron transport

chain has been implicated which is capable of transferring electrons from a reduced pyridine nucleotide to oxygen (Carr, 1973a). It has been suggested that NADP, produced by means of the oxidative pentose phosphate pathway, and not NAD is the source of reductant participating in respiratory electron transfer (Leach and Carr, 1968; Biggins, 1969) and that oxidative phosphorylation, resulting in the production of ATP, seems to be coupled with this process (Biggins, 1969; Leach and Carr, 1970). These organisms seemed to be relatively insensitive to cyanide, azide and antimycin A so it has been suggested that the terminal oxidase in the respiratory chain is an o-type cytochrome rather than the a-type cytochrome found in higher organisms (Webster and Hackett, 1966; Leach and Carr, 1970).

Light has been found to inhibit respiration in cyanobacteria indicating that photosynthesis and respiration compete for substances and sites which are common to both reactions (Fogg et al., 1973). Jones and Myers (1963) suggested that the photoinhibition of respiration in A. nidulans could be the result of competition for electrons between chlorophyll a and the cytochrome oxidase. However, it has since been found that glucose-6-phosphate (G6P) dehydrogenase, the initial enzyme of the pentose phosphate pathway, was allosterically inhibited by RuBP which is the only sugar phosphate unique to the Calvin cycle (Pelroy et al., 1972). Consequently, it was assumed that

during active photosynthesis the pool level of RuBP would be sufficiently high to prevent the activity of G6P dehydrogenase, although when the illumination was removed the RuBP pool would be rapidly depleted and the allosteric inhibition of G6P dehydrogenase relieved, leading to a rapid conversion of the substrate to 6-PG. It was therefore concluded that the photoinhibition of respiration in these organisms was due to an indirect effect of the inhibition of G6P dehydrogenase by RuBP which blocked the transfer of electrons to NADP from the oxidizable substrates of the pentose phosphate pathway, G6P and 6-PG, so preventing the electron flow to oxygen (Stanier, 1973).

In principle, the primary oxidation of G6P through the oxidative pentose phosphate pathway could be coupled with the terminal oxidation of pyruvate through the reactions of the TCA cycle (Stanier and Cohen-Bazire, 1977). However, it seems that the TCA cycle cannot function as a respiratory pathway in cyanobacteria since it has been found that these organisms did not synthesise α-ketoglutarate dehydrogenase and succinyl-CoA synthetase (Pearce and Carr, 1967b; Smith et al., 1967), although all the other TCA cycle enzymes were found to be active (Pearce, Leach and Carr, 1969). Further evidence of an incomplete TCA cycle has been obtained due to the incorporation of radiocarbon from [^{14}C]-acetate mainly into the lipid fraction and to a smaller extent into the protein fraction of cyanobacterial cells (Hoare,

Hoare and Moore, 1967; Smith et al., 1967; Hoare, Hoare and Smith, 1969). These workers showed that only four amino acids, glutamate, proline, arginine and leucine, contained a significant amount of radioactivity indicating that the cycle was interrupted at the level of α-ketoglutarate oxidation (figure 1.2.). Therefore, this cycle seems to have a purely biosynthetic role in these organisms and, in order to produce the aspartate family of amino acids, the reaction sequence from succinate to oxaloacetate must operate in reverse (Smith et al., 1967). It is possible that the synthesis of oxaloacetate needed for these reactions could be the result of the carboxylation of pyruvate or PEP (Smith et al., 1967; Pearce et al., 1969).

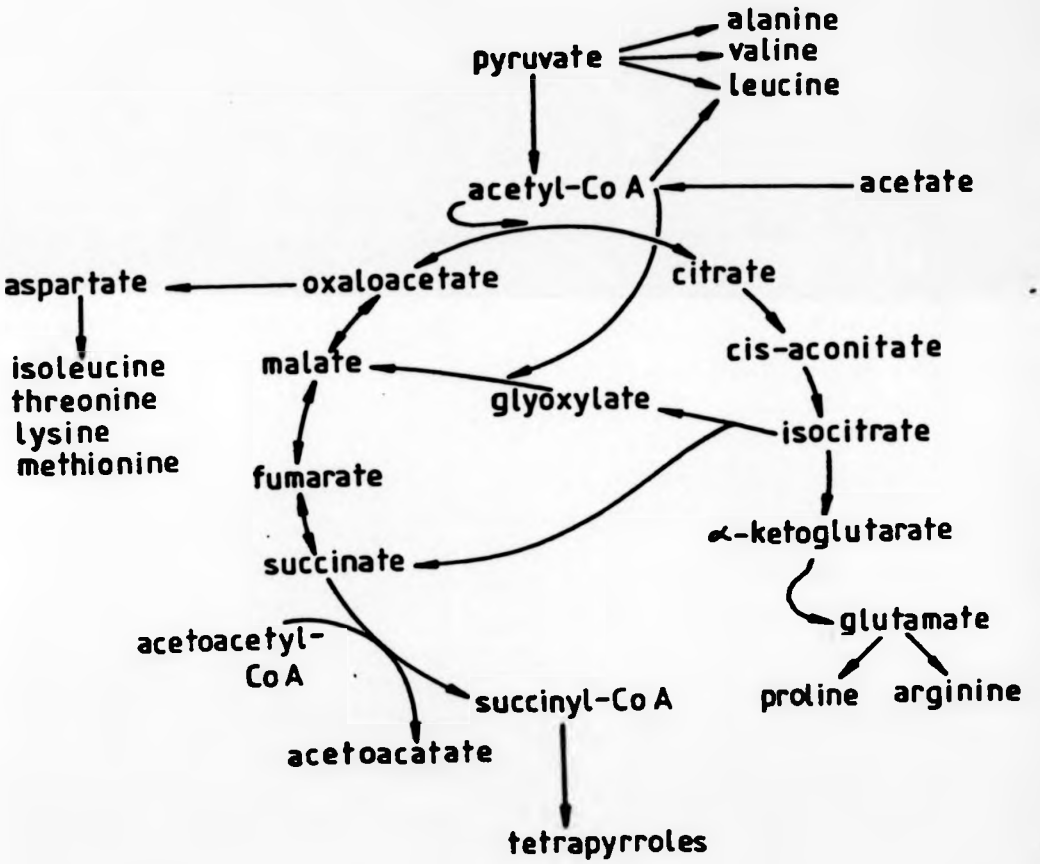
Pearce and Carr (1967a) detected isocitrate lyase and malate synthetase in low concentrations in extracts of A. nidulans and A. variabilis indicating that the glyoxylate shunt may permit a slow flow of carbon from isocitrate to succinate and further towards the synthesis of tetrapyrrole compounds (figure 1.2.). Also, Pearce et al., (1969) found that succinyl-CoA, which is necessary for tetrapyrrole synthesis, was formed in extracts of A. variabilis from succinate by β-ketoacyl-CoA transferase using acetoacetyl-CoA as the CoA donor.

As the oxidative pentose phosphate pathway is the sole energy yielding dissimilatory pathway in these organisms, the range of organic compounds which can support growth is very narrow with only those exogenous substrates readily convertible

Figure 1.2.

The incomplete tricarboxylic acid cycle in cyanobacteria

(Redrawn from Smith et al., 1967; Fogg et al., 1973)


alanine
valine
leucine
pyruvate
acetyl-Co A
acetate
aspartate
oxaloacetate
citrate
isoleucine
threonine
lysine
methionine
malate
cis-aconitate
glyoxylate
isocitrate
fumarate
α-ketoglutarate
succinate
glutamate
acetoacetyl-
Co A
proline
arginine
succinyl-Co A
acetoacatate
tetrapyrroles

to G6P able to support growth in the dark (Stanier, 1973). A number of cyanobacteria were able to grow as facultative chemoheterotrophs in the dark on a limited range of sugars, such as glucose, sucrose, fructose, ribose and maltose, for example, C. fritschii (Fay, 1965), Noctoc muscorum (Kratz and Myers, 1955; Lazaroff and Vishniac, 1961), Aphanocapsa 6714 (Rippka, 1972), P. boryanum (White and Shilo, 1975) and various species tested by Allen (1952) and Khoja and Whitton (1971). However, growth under these conditions was generally very much slower than in the light and there was little increase in the respiration rate. It is probable that the rate of growth is always limited by the rate of dark ATP synthesis which is always far less by non-photochemical means than by photophosphorylation (Stanier, 1973). Other suggestions for the reduced heterotrophic growth rates include permeability problems (Pelroy et al., 1972), lack of transcriptional control (Carr, 1973a) and inefficient control at the level of gene expression (Raboy, Padan and Shilo, 1976).

Some cyanobacteria, although unable to grow chemoheterotrophically could grow photoheterotrophically, for example, A. quadruplicatum and Lyngbya lagerheimii (van Baalen, Hoare and Brandt, 1971) and Aphanocapsa sp. (Rippka, 1972). On the other hand, many cyanobacteria, including A. nidulans, seem to be obligate photoautotrophs even though radioactive tracer techniques have clearly shown that these organisms could assimilate exogenously supplied organic compounds in the light (Kratz and Myers, 1955; Hoare et al.,

1967; Pearce and Carr, 1967a; Smith et al., 1967; Hoare et al., 1969; Pearce and Carr, 1969). These organisms could metabolise these organic substrates via the normal routes but neither the growth nor the respiratory rate was shown to be increased so it seemed that carbon dioxide was still being used as the major source of cell carbon despite the incorporation of potential metabolites (Carr et al., 1969).

It can be concluded that as all cyanobacteria so far examined use light as an energy source and carbon dioxide as a carbon source, their dominant nutritional mode is photoautotrophy (Stanier, 1973). However, these organisms are able to assimilate organic compounds which may play secondary although important roles in their photometabolism.

1.2. EFFECT OF ENVIRONMENTAL CONDITIONS ON MACROMOLECULAR COMPOSITION

1.2.1. Total macromolecular composition

Collyer and Fogg (1955) showed that cyanobacteria contain, on average, on a dry weight basis (w/w), 50% protein, 30% carbohydrate, 5% lipid and 15% ash. Other typical analyses of marine and freshwater species (Parsons, Stephens and Strickland, 1961; Holohan and Moore, 1967) showed the following composition on a dry weight basis(w/w), 20-45% protein, 30-55% carbohydrate, 15% lipid, 2% RNA, 0.4-0.8% DNA and 1.5% total pigment. Environmental factors, such as the composition of the growth medium, can greatly influence the composition of cyanobacterial cells. For example, van Baalen and Marler (1963) found that nitrate-grown cells of

the marine cyanobacterium Agmenellum quadriplaticum contained on a dry weight basis (w/w), 64% protein, 27% carbohydrate and 9% lipid. However, when the same organism was grown with uric acid as the nitrogen source, very different results were obtained, the cells containing on a dry weight basis (w/w), 18% protein, 74% carbohydrate and 7% lipid. It was concluded that these differences were due to the fact that this organism seemed to have a limited ability to degrade uric acid adequately so that under these conditions the cells were, in effect, nitrogen deficient. This would explain the large decrease in protein content and the increase in carbohydrate content which was presumably the main reserve product in organisms grown under these conditions.

Herbert (1961) found that the chemical composition of heterotrophic bacteria also changed according to environmental conditions. It was concluded that, of the major cell constituents, the protein content varied least, undergoing variations of 50 to 100%, the DNA content could vary by 2-3 fold, while the RNA, polysaccharide and lipid contents could vary by 10 fold or more. Growth conditions are therefore very important in determining the macromolecular composition of an organism so it is obviously necessary to carry out such determinations under highly controlled environmental conditions.

1.2.2. Nucleic acid and protein composition

The effects of growth rate and growth environment on the

macromolecular composition, in terms of DNA, RNA and protein, of heterotrophic microorganisms have been extensively investigated (Schaechter, Maaløe and Kjeldgaard, 1958; Herbert, 1961; Maaløe and Kjeldgaard, 1966; Skjold, Juarez and Hedgcoth, 1973; Dennis and Bremer, 1974). It was found, using both batch and continuous culture techniques with several bacterial species, that the DNA, RNA and protein contents on a per cell basis all increased with increasing growth rate, with the RNA per cell increasing more rapidly with growth rate than the DNA and protein contents per cell which tended to increase in proportion to each other. As a consequence of this, on a percentage dry weight basis the RNA content was found to increase with increasing growth rate while the % DNA and protein contents decreased.

Slightly different results were obtained by Aiking and Sojka (1979) working on the photosynthetic non-sulphur purple bacterium Rhodopseudomonas capsulata, who found that on a percentage dry weight basis the RNA content increased with increasing growth rate and the % DNA content increased very slightly. The protein content also increased up to a dilution rate of 0.244 h^{-1} and then decreased with further increase in growth rate.

There has, so far, been little work carried out on the effect of environmental conditions on the macromolecular composition of cyanobacteria. The effects of growth rate, different rates being obtained by altering the incident light intensity, on the DNA and RNA composition of the filamentous

cyanobacterium A. variabilis and the unicellular cyanobacterium A. nidulans have been studied by Leach, Old and Carr (1971) and Mann and Carr (1974) respectively. It was shown, using batch growth techniques, that both the DNA and RNA contents of these organisms on a per cell basis increased exponentially with increasing growth rate . Furthermore, in contrast to the results obtained for heterotrophic bacterial growth, the DNA to RNA ratio remained constant over the growth rate range examined. In the case of A. nidulans an approximate seven-fold increase in both DNA (5.5 to 37 fg $(cell)^{-1}$) and RNA (33 to 230 fg $(cell)^{-1}$) was found over a growth rate range of 0.04 to 0.30 h^{-1}. A comparable value for the DNA content of A. nidulans of 30 fg $(cell)^{-1}$ (equivalent to 1.7% of the dry weight) was obtained by Craig, Leach and Carr (1969).

In contrast to the results obtained for A. nidulans, studies on the DNA content per cell of log phase cultures of the marine cyanobacterium A. quadruplicatum with doubling times of 7 and 20 hours both gave similar average values of 8.6×10^9 daltons (Roberts, Klotz and Loeblich, 1977). If it is assumed that the cell size increased with increasing growth rate then these results would mean that the DNA content on a dry wt. basis decreased.

The effects of growth rate and growth environment on the protein content of A. nidulans have also been studied. Slater (1975) found that the protein content on a per cell and a dry weight basis, under light-limited continuous culture conditions,

increased with increasing growth rate over the growth rate range 0.02 to 0.10 h^{-1}. Karagouni (1979) found that under both light- and carbon dioxide-limited continuous culture conditions, the protein content on a per cell and dry weight basis tended to decrease with increasing growth rates above approximately 0.08 h^{-1}.

Tindall, Yopp, Schmid and Miller (1977) found that the composition of the growth medium as well as the stage of growth affected the protein content of the marine cyanobacterium A. halophytica. The highest protein content of 76% (w/w) was obtained for stationary phase organisms grown in medium containing 1M NaCl, with younger cells and cells grown in higher NaCl concentrations showing a decreased protein content down to a minimum value of 43% (w/w).

1.2.3. Pigment composition

There have been numerous studies on the effects of different environmental conditions on the pigment composition of cyanobacteria in terms of chlorophyll a, phycobiliproteins and carotenoids. The phycobiliproteins, the principal photosynthetic accessory pigments in these organisms (section 1.1.1.), usually represented 1-10% of the dry weight of the cells (Chapman, 1973) although under certain circumstances the value may be as high as 24%, as in A. nidulans (Myers and Kratz, 1955), and can account for 40-60% of the soluble protein content (Myers and Kratz, 1955; Fay, 1969; Bogorad, 1975; Glazer, 1977; Stanier and Cohen-Bazire, 1977). The phycobiliproteins are, therefore, major products of

protein synthesis and energy utilisation and it is clearly of selective advantage for these organisms to control the rates of synthesis of the phycocyanin and phycoerythrin apoproteins (and their cognate chromophores) in ways that optimize the functional phycobiliprotein content for any environmental condition. The phycobiliprotein content, as well as the chlorophyll content, of cyanobacteria is affected by a number of environmental factors, including light quality and quantity, carbon dioxide availability, aging, temperature and mineral nutrition.

Myers and Kratz (1955) and Allen (1968a) found that the concentrations of chlorophyll a and phycocyanin in A. nidulans decreased with increasing light intensity, increasing growth rate and decreasing temperature although the ratios of these two pigments showed only minor changes. The total carotenoid content was found to show little variation at all. Allen (1968a) also found that the lamellar content varied inversely with the light intensity, the amount of double membrane being directly proportional to the chlorophyll content of the cells. It has been suggested that more pigment was present at lower light intensities in order to allow maximum light capture (Krogmann, 1973).

Halldal (1958) grew A. nidulans and Anabaena sp. on agar in crossed gradients of light intensity and temperature and showed similar trends of pigment concentration, except that for

A. nidulans growing at the high temperatures of 42-45°C, both the phycocyanin and chlorophyll contents increased at higher light intensities. In contrast, however, it was shown that the carotenoid content of the cells changed with light intensity and temperature with the relative amounts of carotenoids within the growth area being consistently high where the relative amounts of chlorophyll and phycocyanin were low and vice versa. It was suggested that the yellow pigments might protect the photosynthetic apparatus from the damaging effects of high light intensities.

In contrast to the above results, Ihlenfeldt and Gibson (1975a) observed no changes in the chlorophyll or carotenoid content with increasing light intensity in another strain of A. nidulans although they did observe an inverse relationship between light intensity and the phycocyanin content at light intensities resulting in half maximal growth rates or above. Brown and Richardson (1968) found that the phycocyanin/chlorophyll ratio tended to decrease with increasing light intensity in several cyanobacterial and red algal species.

Light intensity was also found to affect the pigment content of photosynthetic bacteria and higher algae. Aiking and Sojka (1979) working on the non-sulphur purple bacterium R. capsulata grown under chemostat continuous culture conditions, found that for a light-limited culture at a constant dilution rate, regardless of the light intensity, the bacteriochlorophyll and carotenoid concentrations remained constant. It was concluded that the

increased illumination incident upon the culture at a given dilution rate served only to increase the steady state culture density and so supported the idea that self-shading resulted in all the cells receiving the same actual number of photons irrespective of the light intensity. On the other hand, when the illumination was held constant and the growth rate increased a similar result was obtained to that of A. nidulans with a decrease in the bacteriochlorophyll and carotenoid contents and the bacteriochlorophyll/carotenoid ratio remaining constant. (The carotenoids acting as the principal accessory pigments in this organism as bacteria do not contain any phycobiliproteins).

Similar results were also obtained for the non-sulphur purple bacteria R. spheroides (Cohen-Bazire, Sistrom and Stanier, 1957) and Rhodospirillum molischianum (Gibbs, Sistrom and Worden, 1965) in which the bacteriochlorophyll and carotenoid contents were inversely related to the light intensity and directly related to temperature. However, the bacteriochlorophyll/carotenoid ratio was found to decrease with increasing light intensity. From electron microscopy studies it was found that the total amount of membrane (cell membrane plus internal membrane) was directly proportional to the bacteriochlorophyll content (Gibbs et al., 1965). A comparable decrease in the chlorophyll and carotenoid content with increasing light intensity and a decrease in the chlorophyll/carotenoid ratio was shown in the green alga E. gracilis (Cook, 1963). On the other hand, chlorophyll b

(the major accessory pigment in this organism) changed in parallel to chlorophyll a. Treharne, Melton and Roppel (1964) showed a similar increase in the number and density of lamellae in C. pyrenoidosa under low light conditions with a concommittant increase in the chlorophyll content of these cells compared with organisms grown at high light intensities. Also, Myers (1946) found that the chlorophyll content of Chlorella sp. on a dry weight basis was inversely related to the light intensity but on a per cell basis the chlorophyll content was essentially constant over a range of intensities from 60-3600 lux due to a direct relationship between the intensity of light and the cell size.

An extensive study was carried out on the pigmentation of A. nidulans by Goedheer (1976) who found that this organism when grown under high illumination had different mutual ratios of chlorophyll, phycocyanin and carotenoids depending on the environmental conditions during growth. It was observed that sufficient CO_2 availability and growth temperatures between 30 and 38°C resulted in a phycocyanin/chlorophyll ratio similar to that of cells grown under low light intensities (blue-green cells), whereas a deficiency of CO_2 or low growth temperatures led to a low phycocyanin/chlorophyll ratio (green cells) and high growth temperatures (40-45°C) led to a high phycocyanin/chlorophyll ratio (blue cells). These results confirmed the work of Eley (1971) who found a higher phycocyanin/chlorophyll ratio in cells of A. nidulans grown in air enriched with 1% (v/v) CO_2 than in plain

air (0.03% (v/v) CO_2), the total pigment content decreasing from 20.5% to 11.1% of the dry weight of the cells respectively. This decrease was almost entirely due to a decrease in phycocyanin, there being little change in the chlorophyll a and carotenoid contents. It was suggested that the pigmentation, controlled by the CO_2 concentration acting through phycocyanin, might allow adjustment of the light reactions of photosynthesis to match the dark reactions (air grown cells being limited by the availability of CO_2 for dark reactions) by which CO_2 is fixed and might do so without upsetting the balance between the two photoreactions. A similarly decreased phycocyanin/chlorophyll ratio was observed by Goedheer and Kleinen Hammans (1977) for A. nidulans grown in air rather than in CO_2 enriched air.

Goedheer (1976) did not, however, find any influence of the spectral composition of light during the growth of A. nidulans and found that 'blue cultures' could be obtained at temperatures above 40°C with a light source which lacked red or far red radiation. In contrast, Jones and Myers (1965) found that the growth of A. nidulans in wavelengths of light predominately absorbed by chlorophyll a caused a dramatic lowering of the chlorophyll content with only small changes in the phycocyanin and carotenoid contents. The chlorophyll/phycocyanin ratio was found to decrease with increasing wavelength from 619 to 680 nm and with decreasing wavelength from 560 to 436 nm. Also, a four-fold decrease in chlorophyll content was observed with only a slight increase in phycocyanin induced by special red light

illumination. It was assumed that the mechanism was working to prevent pigment system I from receiving quanta in excess (the bulk of chlorophyll a collecting for PSI with phycocyanin being the principal absorber for PSII) and, therefore, maintaining the balance of energy input to both pigment systems. Similar results were obtained by Myers, Graham and Wang (1978) for the growth of A. nidulans at different wavelengths of light and also two other Synechococcus strains showed a severely reduced chlorophyll/phycocyanin ratio when grown in far red light. However, this dramatic response was not shown by A. quadruplicatum and Anabaena sp. which showed only a minor decrease in the pigment ratio. It was suggested that far red illumination was an exotic condition not likely to occur in nature and, therefore, it was possible that the low chlorophyll condition arose as an incompetence and should be judged as a failure rather than an adaptation. Jones and Myers (1965) did indeed find that only small variations in the chlorophyll/phycocyanin ratio occurred under white illumination and so it was concluded that no serious change should occur in this ratio by any illumination likely to be experienced in nature.

Similar results were obtained for A. nidulans by Ghosh and Govindjee (1966) who found that, in general, the proportion of the pigment which best absorbed the light supplied during the growth period was reduced when strong light was used. However, the colour of weak light did not significantly affect the proportions of the pigments. It was found that organisms grown in red light of high intensity (absorbed mainly by chlorophyll) contained relatively

more phycocyanin whereas cells grown in orange light of high intensity (absorbed mainly by phycocyanin) contained relatively more chlorophyll a. In agreement with other previous work it was also found that the total pigment concentration was low in all cells which were grown at high light intensities.

A similar effect to culturing A. nidulans in far red light was shown in manganese deficient cells (manganese acting as a catalyst in PSII) (Cheniae and Martin, 1968). Manganese deficiency in A. nidulans, in contrast to the very minor effects on the pigmentation of the green alga Scenedesmus, led to an excessive loss of chlorophyll a and a slight loss of phycocyanin. This was concluded to be an adaptive effect to maintain the balance between the pigments associated with photosystems I and II.

Another type of chromatic effect has been shown in some cyanobacteria which contain the phycobiliprotein phycoerythrin, as well as phycocyanin, and this phenomenon is generally known as complementary chromatic adaptation. (Tandeau de Marsac (1977) found that not all phycoerythrin-containing cyanobacteria could adapt chromatically). In white light the organisms contained nearly equal amounts of the two phycobiliproteins but in orange or red light phycocyanin synthesis was most strongly induced and in blue or green light phycoerythrin synthesis was most strongly induced (Hattori and Fujita, 1959; Bennett and Bogorad, 1973; Glazer, 1977; Tandeau de Marsac, 1977). Therefore, as a consequence of this phenomenon, the pigment which absorbs the incident wavelengths of light most strongly becomes dominant so that there is maximum utilisation of available light energy for photosynthetic purposes.

This chromatically induced modification of the phycocyanin/ phycoerythrin ratio was found to involve de novo protein synthesis, not turnover (Bennett and Bogorad, 1973) and according to Tandeau de Marsac (1977) the adaptation involved light induced modulation either of phycoerythrin synthesis alone or both phycoerythrin and phycocyanin synthesis depending on the strain concerned.

Chromatic adaptation was found to be largely independent of light intensity and accompanying changes in chlorophyll and carotenoid contents were not found in Tolypothrix tenuis (Hattori and Fujita, 1959) nor in the cellular chlorophyll a content of a number of cyanobacterial strains (Tandeau de Marsac, 1977). However, the phycobiliprotein/chlorophyll a ratio in Fremyella diplosiphon adapted to red illumination was twice that in cultures adapted to fluorescent illumination (Bennett and Bogorad, 1973).

A similar adaptation was found by Rai (1977) who observed that although phycoerythrin was not present in Anabaena ambigua under normal culture conditions, it could be induced in the parent and in one of its mutant strains when treated with green light and nitrate. This adaptation is, in fact, a natural phenomenon since cyanobacteria which occur in deep water usually show chromatic adaptation (Fogg et al., 1973). For example, the marine cyanobacterium Lyngbya sordida, which in surface illumination is blue-green or brownish in colour, is pink when growing at a depth of 20-30 m because of a preponderance of phycoerythrin. This pigment has an absorption maximum in the blue-green, the wavelengths that are best transmitted by clear water, so that this adaptation presumably increases the photosynthetic efficiency of the alga in deep water.

Temperature, as well as light, was found to have an effect on the pigmentation of A. nidulans. The phycocyanin and chlorophyll a contents were found to decrease with decreasing temperature with little change in the carotenoid content (Myers and Kratz, 1955; Allen, 1968a). Sherman (1978) found a higher phycocyanin/chlorophyll ratio at 40°C than 30°C in Synechococcus cedrorum which is in agreement with the results of Goedheer (1976) who obtained 'blue cells' of A. nidulans at high growth temperatures of 40 - 45°C. If the growth temperature for A. nidulans was increased above 45°C, however, the culture bleached and died with a similar result at very low temperatures (Halldal, 1958; Goedheer and Kleinen Hammans, 1977). A similar chlorosis of A. quadruplicatum was obtained due to culture aging which was attributed to progressive depletion of inorganic nitrogen from the culture medium following exponential growth of the organism and was caused by a consecutive loss of phycocyanin and chlorophyll and a smaller decline in the total carotenoid content, although the proportions of the major carotenoids remained remarkably constant (Antia and Cheng, 1977). Goedheer (1976) obtained similar results for the aging of A. nidulans cultures and found that the original pigmentation and growth was resumed when the cells were resuspended in fresh culture medium.

A number of investigations have been carried out on the effects of nitrogen chlorosis due to nitrogen starvation in cyanobacteria. Allen and Smith (1969) found no phycocyanin but only slight variations in the levels of chlorophyll and carotenoids in nitrogen-deficient cultures of A. nidulans. It was, therefore, suggested that the phycocyanin was acting as an emergency nitrogen reserve in the

cells, a theory that has been reinforced by the work of van Gorkom and Donze (1971) and DeVasconcelos and Fay (1974). Several workers have shown a decrease in the chlorophyll content, as well as the phycocyanin content, due to nitrogen starvation, such as in A. nidulans (Lau, MacKenzie and Doolittle, 1977) and in A. cylindrica (DeVasconcelos and Fay, 1974), which led to a yellowing of the cultures as the non-nitrogen containing carotenoid pigments persisted and the nitrogen containing pigments were utilised. Other nitrogen-deficient storage products, lipid and glycogen, were also found to accumulate under nitrogen-limitation (DeVasconcelos and Fay, 1974; Goedheer and Kleinen Hammans, 1977). Lau et al. (1977) suggested that nitrogen starvation specifically depressed the expression of the phycocyanin structural genes preventing further de novo synthesis. The addition of a utilisable nitrogen source in all of these experiments led to the recovery of normal growth and pigmentation.

The specific phycocyanin content of A. nidulans was also sharply reduced during phosphate starvation while the chlorophyll content remained relatively stable and the carotenoid content increased leading to yellowish-green cells (Ihlenfeldt and Gibson, 1975b). Addition of phosphate led to a return to normal colouration. It was concluded that changes in the specific content of phycocyanin appear to be a sensitive indicator of starvation conditions in A. nidulans.

These studies seem to indicate that photosynthetic organisms in general can change their pigmentation levels in response to various environmental conditions to allow maximum light capture

and maintain the balance of quanta input into the two photoreactions.

1.2.4. Carbohydrate composition

The major reserve polysaccharide of cyanobacteria is a polymer of glucose with a degree of branching between glycogen and amylopectin which cytochemically resembles glycogen (Fuhs, 1973). In bacteria and yeasts the glycogen content (also the most important of the intracellular polysaccharides in these organisms) has been reported to vary greatly with values ranging from 2% (w/w) to over 30% (w/w) for the same organism under different growth conditions (Herbert, 1961).

Storage compounds, such as glycogen and also lipids, may provide both a source of carbon and energy. To be designated as a storage compound there are a number of criteria a compound has to meet: it must be accumulated under conditions when the supply of energy from exogenous sources is in excess of that required by the cell for growth and related processes; it must be utilised when the supply of energy from the exogenous sources is no longer sufficient for optimal maintenance of the cells, either for growth and division or for maintenance of viability and other processes; and it must be degraded to produce energy in a form utilisable by the cell which is utilised to give a biological advantage in the struggle for existence over cells which do not have a comparable compound (Dawes and Senior, 1973).

Parsons et al. (1961) found that carbohydrate was the main storage product in A. quadruplicatum amounting to 31.5% of the total dry weight of the organism with glucose being the predominant sugar and galactose, fructose, ribose, hexosamine and hexuronic

acids being present in smaller amounts. A previous study by Norris, Norris and Calvin (1955) showed the presence of sucrose and glucose in Phormidium sp. and N. muscorum and sucrose in Nostoc sp., with N. muscorum also containing ribulose and possibly fructose plus small amounts of several other sugars.

Generally, it seems that microorganisms which accumulate glycogen do so under conditions where growth is limited by the supply of utilisable nitrogen and there is a plentiful supply of exogenous carbon. Under nitrogen-limiting conditions, as would be expected, nitrogen-deficient storage products, such as glycogen and lipid, tend to accumulate, whereas nitrogen-containing compounds, such as proteins and pigments, tend to decrease (De Vasconcelos and Fay, 1974; Goedheer and Kleinen Hammans, 1977). This was shown by the results of Allen and Smith (1969) who found that growth on media containing limiting amounts of a combined nitrogen source resulted in a three-fold increase in the polysaccharide content of A. nidulans after growth had ceased. Addition of a nitrogen source, however, led to a decrease in the reducing sugar content and recovery of the normal cellular composition. Similarly, nitrate grown cells of A. quadriplaticum contained, on a dry weight basis, 27% carbohydrate, whereas uric acid grown cells, which were effectively nitrogen-limited due to the limited ability of this organism to degrade uric acid to a useful nitrogen source, contained 74% carbohydrate, indicating that polyglucoside material was the main reserve product of uric acid grown organisms (van Baalen and Marler, 1963).

Extensive studies by Lehmann and Wöber (1975; 1976; 1977;

1978a; 1978b) also showed that A. nidulans accumulated glycogen as a carbon reserve during illumination and nitrogen limitation. It was found, using batch grown cultures, that during the post-exponential phase of growth in the presence of an excess of a utilisable carbon source glycogen accumulated, due to nitrogen starvation, up to a constant amount per cell. Conditions of temporary starvation, such as the removal of light and carbon dioxide or the addition of DCMU to an illuminated culture were found to affect the cellular glycogen content (Ishmann and Wöber, 1975; 1976; 1978a). Removal of carbon dioxide, the sole carbon source, while illuminating the cells was followed by a stagnation of the glycogen content, whereas removal of the light source led to a rapid decrease in the amount of α-glucan present in the cells, eventually leading to cell death. When 3-(3,4-dichlorophenyl)-1,1-dimethylurea or DCMU (a specific inhibitor of PSII) was added at the end of exponential growth there was no accumulation of glycogen, whereas the addition of DCMU to cells already containing glycogen caused a mobilization of the α-glucan. These starvation conditions which led to the mobilization of glycogen indicated that the α-glucan was being utilised as a reserve carbon source for the cell. It was also found that A. nidulans having accumulated glycogen by preculture under nitrogen-limiting conditions resumed cell division when the culture medium was complemented with a nitrogen source. A concomitant removal of carbon dioxide from the aeration mixture, however, forced the organism to support an increase in cell number at the expense of glycogen, therefore demonstrating the physiological

function of glycogen as a reserve substance subject to mobilisation when an external carbon source was lacking. The reserve polysaccharide was shown to be subject to turnover, as demonstrated with a pulse-chase labelling technique, even during the time when the amount per cell remained constant, which indicated a strict balance in the relative rates of synthesis and degradation. Another indication of the function of glycogen as the principal storage product in A. nidulans was the finding that this compound was synthesised in the light and degraded in the dark (Lehmann and Wöber, 1978b).

Nitrogen-limitation was found to affect the glycogen content of other microorganisms in a similar manner. Holme and Palmstierna (1956) concluded that glycogen deposition in E. coli B seemed to occur when nitrogen was limiting, with subsequent addition of the limiting substrate leading to a decrease in glycogen content. However, when glucose was limiting, the glycogen content of the cells remained low throughout the growth of the culture. Holme (1957) confirmed these results for E. coli grown under nitrogen-limiting conditions in a steady state continuous-flow culture system, with glucose as the carbon source, finding that the glycogen content increased as the growth rate and nitrogen concentration decreased from approximately 3% of the total dry weight at $D=0.85\ h^{-1}$ to approximately 23% at $D=0.13\ h^{-1}$. Under nitrogen-limitation with concomitant glycogen accumulation, it was found that the synthetic rate of nitrogen-containing compounds, such as protein, was selectively reduced. Identical results were obtained under nitrogen-limiting conditions when lactate replaced glucose as the carbon source in this system.

Similar results were reported for a yeast Torula utilis (Herbert, 1961). The total carbohydrate content of the cells remained virtually

constant under glucose-limited continuous-flow culture conditions but increased with decreasing growth rate when ammonia was limiting. The results suggested that the high glycogen content was a result not of growth at a low rate but of growth in a low concentration of ammonia. In fact, in resting cells of T. utilis, which were nitrogen-limited so that no growth would occur and which were supplied with glucose, carbohydrate was found to accumulate. The cells doubled their dry weight in two hours, an increase which could be entirely accounted for in terms of glycogen synthesised.

Glycogen accumulation was also found to occur due to phosphate- and sulphate-limitation. Dicks and Tempest (1967) found that phosphate- and sulphate-limited organisms of Aerobacter aerogenes contained considerable amounts of polysaccharide, especially when grown at a low rate. However, potassium-limited organisms contained little carbohydrate especially when grown at a low dilution rate where glycogen accumulation would be expected. It was found that the inhibition of glycogen accumulation under these conditions was due to the ammonia concentration, so that the synthesis of glycogen depended on the relative concentrations of extracellular potassium and ammonia, polysaccharide deposition being promoted by potassium and inhibited by ammonia. On the other hand, an increase in the extracellular potassium concentration in the phosphate-limited culture led to a net decrease in the cellular carbohydrate content from 18 to 8% of the dry weight.

Other environmental conditions which affect the carbohydrate content of microorganisms include light intensity and temperature.

In the green alga E. gracilis the paramylum (the carbohydrate reserve in this organism) content changed directly with light intensity being only approximately 5% of the total mass at 65 - 120 ft-candles and 45% at 1200 - 3000 ft-c (Cook, 1963). Paramylum synthesis was also found to increase rapidly with light intensity up to an intensity of 1200 ft-c but then decreased slightly with further increases in intensity. The polysaccharide levels were low between 400 and 1200 ft-c due to decreased photosynthetic rates despite a heightened chlorophyll content. These results suggested that protein synthesis had the first call on energy available to the cell and only when protein synthesis and the multiplication rate were optimal and energy exceeded these requirements did polysaccharide accumulate. Maintenance activities therefore seemed to have a low priority in the apportionment of energy, although protein stores could be used for maintenance in cases of extremely limited energy supplies.

Aiking and Sojka (1979) working on light-limited continuous-flow cultures of R. capsulata found that light-limited cells from cultures with a similar dilution rate had a similar carbohydrate composition regardless of the incident light intensity. This supported the conclusion that self-shading resulted in all cells receiving the same actual number of photons irrespective of the light intensity. On the other hand, when grown at constant illumination the carbohydrate composition was found to vary with the dilution rate increasing up to a peak at the lower dilution rates and then decreasing with further increases in growth rate.

Temperature has been found to affect the carbohydrate content

of a number of microorganisms, there being a tendancy to accumulate greater quantities of polysaccharides at suboptimal temperatures (Farrell and Rose, 1967). Tempest and Hunter (1965) working on continuous cultures of A. aerogenes found a significant increase in the carbohydrate content on a dry weight basis with decreasing temperature from 2.9 to 8.9% under glycerol-limitation and from 2.1 to 4.3% under magnesium-limitation. A significant increase in carbohydrate content was also shown under both limitations at low pH values although at higher values the content was almost constant. It was not, however, determined whether the extra carbohydrate at the low temperatures was present as a storage compound, such as glycogen, or resulted from an increase in the structural, cell wall, polysaccharide material.

1.2.5. Lipid composition

The major lipid components in cyanobacteria are four fatty acid containing acyl lipids: the phospholipid, phosphatidyl glycerol, and the glycolipids, monogalactosyl diglyceride, digalactosyl diglyceride and sulphoquinovosyl diglyceride (Nichols, Harris and James, 1965; Hirayama, 1967; Fogg et al., 1973; Nichols, 1973). In this respect the cyanobacteria resemble green algae and higher plants, these lipids being found in the chloroplasts of higher photosynthetic organisms. However, they differ in not containing lecithin, phosphatidyl ethanolamine or phosphatidyl inositol and in this respect resemble photosynthetic bacteria.

The fatty acid composition of cyanobacteria has been extensively studied and it has been found that, in contrast to

eukaryotic algae, these organisms are heterogeneous with respect to their fatty acid composition, with a tendancy for the more highly unsaturated acids to be found in the morphologically more complex strains. In the majority of unicellular cyanobacteria polyunsaturated fatty acids were found to be absent or in very low concentrations (Holton, Blecker and Onore, 1964; Nichols et al., 1965; Hirayama, 1967; Parker, van Baalen and Maurer, 1967; Holton, Blecker and Stevens, 1968; Kenyon and Stanier, 1970; Stanier et al., 1971; Kenyon, 1972; Fork, Murata and Sato, 1979; Sato, Murata, Miura and Ueta, 1979) and in this respect resemble the bacteria which contain only saturated and monounsaturated fatty acids (Wood, Nichols and James, 1965; Constantopoulos and Bloch, 1967a). On the other hand, the presence of large quantities of polyenoic fatty acids are characteristic of most filamentous cyanobacteria (Nichols et al., 1965; Parker et al., 1967; Holton et al., 1968; Kenyon and Stanier, 1970; Kenyon, Rippka and Stanier, 1972; Sato et al., 1979) and in this respect resemble eukaryotic organisms. There are exceptions to these generalisations. For example, some unicellular species contained large amounts, up to 61% (w/w) of polyunsaturated fatty acids (Kenyon and Stanier, 1970; Stanier et al., 1971; Kenyon, 1972) whereas the morphologically complex Haplosiphon laminosus contained only monounsaturated fatty acids (Holton et al., 1968). Kenyon (1972) even reported that A. nidulans contained small quantities of linoleic acid (18:2) and sometimes of linolenic acid (18:3) depending on the conditions of growth whereas it has been reported on numerous occasions to contain no polyenoic fatty acids

(Holton et al.. 1964; Nichols et al., 1965; Hirayama, 1967; Parker et al., 1967; Sato et al., 1979).

Apart from the acyl lipids, the pigments, chlorophyll a and carotenoids (section 1.2.3.) constitute quantitatively a major part of the lipid extracts from cyanobacteria. Therefore, it would be expected that much of the lipid substances extracted from these organisms originate from the thylakoids and Hirayama (1967) found that the lipid composition of thylakoids isolated from A. nidulans was almost identical to that obtained for the whole cell.

Other lipid species found in cyanobacteria include tocopherols (Botham and Pennock, 1971; DaSilva and Jensen, 1971) although no detectable quantities have been found in A. nidulans (Hirayama, 1967); certain quinones (Carr, Exell, Flynn, Hallaway and Talukdar, 1967); sterols (Fogg et al.. 1973; Nichols, 1973); and hydrocarbons (Nichols, 1973). The common bacterial storage lipid material, poly-β-hydroxybutyrate (PHB), which has a role as a carbon and energy reserve in a large number of photosynthetic and non-photosynthetic bacteria (Doudoroff and Stanier, 1959; Dawes and Senior, 1973) was also found in the cyanobacterium C. fritschii after growth with sodium acetate and amounted to 10% of the total dry weight of the organism, although this material was not formed by A. variabilis under similar growth conditions (Carr, 1966).

It has been found that the lipid composition of microorganisms is very responsive to changes in the chemical and physical properties of the environment, especially growth temperature and composition of the growth medium. As the bulk of the cell lipids in most

microorganisms is in the membranes, it is likely that any environmentally induced changes in the lipid composition are of major physiological importance.

Increasing temperature seems to affect the fatty acid composition in particular. Holton et al. (1964) found that in A. nidulans the saturated fatty acids predominated at 41°C compared with lower temperatures and that growth at 26°C resulted in acids of slightly shorter average carbon chain length than at 32°C and above. Similar, although more pronounced, changes in the fatty acid composition of S. cedrorum grown at 30 and 40°C were shown by Sherman (1978) who concluded that membranes formed by growth at 40°C had fatty acids with a higher melting point and should therefore be more fluid in the temperature range used. Sato et al. (1979) found that the actual lipid composition of the four acyl lipids was affected by growth temperature in A. nidulans but not in A. variabilis, as well as finding changes in the fatty acid composition of both organisms with changing temperature. In A. nidulans the digalactosyl diglyceride content was found to be approximately twice that in cells grown at 38°C as in cells grown at 22°C while the amounts of monogalactosyl and sulphoquinovosyl diglyceride were lower with the amount of phosphatidyl glycerol remaining almost constant. Comparable changes were also found in the fatty acid composition of the thermophilic cyanobacterium Synechococcus lividus with the ratio of unsaturated to saturated fatty acids being 0.31 for cells grown at 55°C which increased over four times to 1.31 in cells grown at 38°C (Fork et al., 1979). The ratio of C_{16} to C_{18} fatty acids in the total lipids was also

found to increase when the growth temperature was lowered.

On the other hand, Kenyon (1972) found in a number of unicellular cyanobacteria that the cellular complement of fatty acids was a strain (or species)-specific property little affected by changing growth conditions. Temperature, light source nor medium composition significantly affected the qualitative nature of the major fatty acids of any of the five strains examined - the only changes observed being quantitative and fairly minor. The expected increase in polyunsaturated acid content at a lower temperature was seen only in the case of strain 6714 with 40% unsaturated fatty acids at 37°C and 58% at 25°C and, in contrast, in strain 6301 (A. nidulans) the percentage of polyunsaturated fatty acids actually decreased from 9% at 37°C to 3% at 25°C.

According to Farrell and Rose (1967) in general when micro-organisms are grown at lower temperatures in their growth range they contain increased proportions of unsaturated fatty acid residues in their lipids (as shown in several cyanobacteria) and it was suggested that temperature directly affects the synthesis or activity, or both, of enzymes that catalyse reactions leading to the synthesis of unsaturated fatty acids.

This expected increase in the degree of unsaturation of the lipids at lower temperatures has been shown in the bacteria E. coli (Marr and Ingraham, 1962) and Serratia marcesens (Kates and Hagen, 1964), the thermophilic eukaryotic unicellular alga Cyanidium caldarium (Kleinschmidt and McMahon, 1970a; 1970b) and the yeast Candida utilis (Brown and Rose, 1969). It was found that the dissolved oxygen tension also affected the fatty acid composition

of C. utilis causing a diminished synthesis of unsaturated fatty acids at a fixed temperature with a decreasing oxygen tension under either glucose-or ammonia-limited chemostat growth (Brown and Rose, 1969). Marr and Ingraham (1962) found that different culture methods gave different results. E. coli cells limited in a chemostat by ammonium salts showing a higher content of saturated fatty acids than cells harvested from an exponentially growing batch culture in the same medium and glucose-limited chemostat grown cells showing a slightly higher content of unsaturated fatty acids than cells from the corresponding batch culture. The total lipid content of C. caldarium cells grown at 55°C was only half of that in cells grown at 20°C and the cells cultured at 20°C contained 30% of their fatty acid as linolenic while this fatty acid was not detected at the higher temperature (Kleinschmidt and McMahon, 1970a; 1970b). It was also shown that cells grown at 55°C were 10 - 15°C more stable to disruption by heating than cells grown at 20°C, the greater thermostability being attributed to the higher degree of saturation of the membrane fatty acids. An increase in the total lipid content of the yeast Saccharomyces cerevisiae was also shown when the growth temperature of batch cultures was lowered (Hunter and Rose, 1972). In contrast to other results for microorganisms little change was found in either the fatty acid composition or the degree of unsaturation of the fatty acids in either batch or chemostat culture grown cells following a change in the incubation temperature.

Nitrogen deficiency is another important factor affecting the lipid composition in many microorganisms. High lipid content,

like high polysaccharide content, tends to be associated with growth in nitrogen-deficient media. This was certainly the case with the major bacterial reserve storage material PHB (Herbert, 1961). In carbon-limited cultures of Bacillus megaterium the PHB content remained low and fairly constant throughout the growth of the culture but in nitrogen-limited cultures there was a rapid synthesis of PHB up to very high levels in the period immediately preceeding and following the cessation of growth due to exhaustion of the nitrogen source. It was found that PHB was also formed in large amounts by resting cells of Bacillus cereus and B. megaterium (Macrae and Wilkinson, 1958) and Pseudomonas saccharophila (Doudoroff and Stanier, 1959) supplied with glucose and certain other substances, such as pyruvate, acetate and butyrate in the absence of any nitrogen source. The latter workers showed that when the external substrate was removed there was a fairly rapid intracellular breakdown of the polymer indicating its storage function. Macrae and Wilkinson (1958) showed that PHB formation was inhibited by high concentrations of oxygen although no synthesis occurred anaerobically in nitrogen. Herbert (1961) concluded that PHB (and perhaps other lipids) resembled glycogen since synthesis was largely controlled by the nitrogen content of the environment. However, it was not certain whether glycogen and PHB were strictly alternative reserve storage materials or whether they could both be formed in the same organism.

Nitrogen deficiency was also found to promote fat accumulation in many eukaryotic algal species (Collyer and Fogg, 1955) although in the Rhodophyceae and Cyanobacteria examined fat accumulation was not associated with low cell nitrogen contents. The proportion

of fatty acids in the Chlorophyceae, Xanthophyceae and Bacillariophyceae species examined increased with age in contrast to the Rhodophyceae and Cyanobacteria species where there was a decrease with age. A similar result was obtained for A. quadriplaticum where effective nitrogen deficiency did not seem to lead to lipid accumulation, with comparable amounts of lipid being present in both uric acid (7%) and nitrate (9%) grown organisms on a dry weight basis (van Baalen and Marler, 1963).

Growth rate was found to affect the lipid content of the green alga Scenedesmus sp. when grown under nitrogen-limiting conditions, the lipid content per cell increasing with increasing growth rate (Rhee, 1978).

Fat accumulation also seemed to be promoted by decreasing water availability (Collyer and Fogg, 1955). Oscillatoria sp. (later reidentified as Microcoleus vaginatus) contained greatly increased amounts of lipids when grown on agar as compared with growth in liquid cultures with an otherwise similar medium. It was suggested that the decrease in chemical potential of water dipoles within the protoplasm would favour an increase in the formation of non-polar groups at the expense of hydrophilic groups.

On the other hand, in S. lividus the lipid content decreased due to carbon dioxide deprivation, it being 30% and 17% of the total dry weight in green (control) cells and bleached (cells in which the pigments had been lost) cells respectively (Miller and Holt, 1977). However, the relative proportions of the four typical acyl lipids were similar indicating no differential loss of one lipid species over another. The green and bleached cells were

also qualitatively similar in fatty acid composition, both cell types containing almost 50% of cellular lipid as palmitate (16:0). The only major difference between the two cell types was the greater amount of a 17 carbon alkane (identified as n-heptadecane) accounting for 23.1% of the analysed material in bleached cells but only 7.5% in green cells. Regreening by the addition of carbon dioxide led to a decreased content of n-heptadecane but a vast increase in the palmitoleate (16:1) content to an amount more than double that in the bleached cells after 48 hours. After 4 days the fatty acid content of the regreened cells was very similar to that of the controls.

The lipid content of microorganisms has also been shown to be affected by light intensity. The total lipid content of E. gracilis being found to decrease with increasing light intensity (Cook, 1963; Constantopoulos and Bloch, 1967b). According to Cook (1963) most of this variation could probably be accounted for by changes in the photosynthetic pigments. Constantopoulos and Bloch (1967b) found that the content of two of the polyunsaturated fatty acids increased sharply with increasing light intensity. A similar result was obtained for Chlorogloea (Holton et al., 1968) in which the triply unsaturated C_{18} acid was formed in the light but only the doubly unsaturated C_{18} acid in the dark. In contrast, Wood et al. (1965) found that changing from light to dark growth usually caused a drop in the amount of saturated acids and an increase in the C_{16} monoene. On the other hand, growth in the dark of a number of heterotrophic filamentous cyanobacteria had little effect at all on the fatty acid content (Kenyon et al., 1972).

1.3. EFFECT OF ENVIRONMENTAL CONDITIONS ON ULTRASTRUCTURE

1.3.1. Fine structure of Cyanobacteria

The cellular organisation of the Cyanobacteria closely resembles that of the bacteria in the absence of a membrane-bound nucleus and other membrane bound organelles and is termed prokaryotic (Stanier and van Niel, 1962; Echlin and Morris, 1965). The cells are characterised by a low level of structural differentiation compared with the eukaryotic organisation of higher organisms, yet functionally they are as highly differentiated, at least in so far as the major processes, such as photosynthesis, respiration and protein synthesis, are concerned. This apparent discrepancy between a high level of physiological and biochemical organisation and a seemingly low level of structural differentiation was an important factor leading to contemporary studies on the fine structure of cyanobacteria (Ris and Singh, 1961; Pankratz and Bowen, 1963; Wildon and Mercer, 1963; Lang, 1968; Fogg et al.. 1973).

The main features of a cyanobacterium as seen under the electron microscope are a prokaryotic cellular organisation, a fibrous sheath, a rigid, multilayered cell wall, an elaborate photosynthetic membrane system, a fibrillo-granular nucleoplasmic region and a diversity of characteristic cytoplasmic inclusions. In these cells the cytoplasmic membrane-containing regions are in direct contact with the regions containing DNA fibrils and ribosomes so that the cell can be considered as a single physiological unit with a close biochemical relationship between subcellular structures of different function (Fogg et al., 1973).

1.3.2. Sheath

Most cyanobacteria have a mucilaginous sheath, composed of pectic acids and acid mucopolysaccharides, although its extent, consistency, pigmentation and structure varies greatly. The sheath, which is often extensive in natural populations and may account for enhanced survival during periods of increasing dessication (Lang, 1968), varies in thickness according to species from being almost undetectable as in Aphanocapsa to thick and striate as in colonies of Gloeocapsa (Fogg et al., 1973). The fibres of the sheath are embedded in an amorphous matrix and show a typical orientation which again varies according to species. For example, the mucilage fibrils of Oscillatoria chalybea which shows a right-handed helical motion comprise a right-handed helical array whereas those of Lyngbya sp. which shows a left-handed helical motion comprise a left-handed helical array round the cylindrical trichome (Lamont, 1969). On the other hand, the array of fibrils may be irregular as in Scytonema julianum and Oscillatoria sancta (Drews, 1973).

According to Fogg et al. (1973) the sheaths often show brown, blue, red and yellow pigmentation which may be dependent on the environmental conditions under which the organisms are growing. Pigmented sheaths are often developed under high light intensities and colourless sheaths under low light intensities, although some organisms never have coloured sheaths at all. Light intensity may also affect the thickness of the sheath. For example, Peat and Whitton (1967) found much wider sheaths surrounding groups of endospores of C. fritschii at low light intensity (550 lux), especially in medium containing nitrogen, although these were less

electron dense than at high light intensity (6500 lux) and Wildon and Mercer (1963) found that the sheath was quite inconspicuous in N. muscorum grown in the light but was very prominent in dark grown cells. Peat and Whitton (1967) also found a very broad sheath in heterotrophic cultures of C. fritschii which had been subcultured for three years in the dark. The thickness of the sheath may also vary with age being thicker in older cells of T. tenuis and F. diplosiphon (Gantt and Conti, 1969).

1.3.3. Cell wall

The cell wall of cyanobacteria is a globular structure which adjoins the plasma membrane (section 1.3.4.) and may be encompassed by a non-globular or fibrous sheath (section 1.3.2.). The cell wall is homologous in structure and composition with that of other Gram-negative prokaryotes (Stanier and Cohen-Bazire, 1977). Under the electron microscope it appears as a multilayered structure (Allen, 1968b; Lamont, 1969; Holt and Edwards, 1972; Wolk, 1973; Golecki, 1977; 1979), the four layers being designated L_I to L_{IV} in an outward order (Jost, 1965). The major cell wall component is a mucopolymer, which is responsible for rigidity and cell shape, composed of two amino sugars, muramic acid and glucosamine, and three amino acids, α-ε-diaminopimelic acid, glutamic acid and alanine, in the molecular ratio of approximately 1:1:1:1:2 (Fogg et al., 1973; Golecki, 1977). A distinctive characteristic of the cell wall due to this mucopolymer is its susceptibility to lysozyme (Crespi, Mandeville and Katz, 1962) and penicillin (Frank, Lefort and Martin, 1962).

1.3.4. Plasma membrane

The plasma membrane maintains the cellular integrity and acts

as a semi-permeable membrane, appearing as a typical tripartite unit membrane approximately 7 nm thick (Ris and Singh, 1961). Jost (1965) investigated the structure by freeze-etching techniques and found that the membrane was composed of globular structures which may have been protein molecules with possible enzymatic functions in respiration and cell wall and sheath formation.

1.3.5. Photosynthetic membrane system

The most extensive cellular structures seen in cyanobacteria with the electron microscope are series of flattened membranous sacs known as thylakoids. Structurally these vesicles appear tripartite having a typical unit membrane structure (Fogg et al., 1973) and have been shown to have a globular structure by freeze-etching techniques (Lang, 1968). The thylakoids are predominately peripheral in some species in which they are generally orientated parallel to the longitudinal cell wall, for example, Synechococcus, Anacystis and Gloeocapsa (Ris and Singh, 1961), Anacystis montana f. minor (Echlin, 1964a), A. nidulans (Echlin, 1964b; Allen, 1968a) and S. lividus (Holt and Edwards, 1972; Golecki, 1979) but their more generally net-like permeation throughout the cytoplasm presents a 3-dimensional maze of interlocking plates (Lang, 1968). The thylakoids contain the photosynthetic pigments, chlorophyll a and carotenoids, (Calvin and Lynch, 1952) and are the sites of photosynthesis. The ability to carry out photophosphorylation, photosynthetic oxygen evolution and the Hill reaction have all been demonstrated in cell-free extracts (Fogg et al., 1973) and they are probably sites of respiratory activity as well (Biggins, 1969).

The thylakoids show considerable variation in arrangement and structure according to the species, physiological state, developmental stage and preparatory procedure. Different methods of fixation may lead to differences in fine structure. For example, when osmium was used as the post-fixative the lamellae appeared as a typical tripartite structure but when permanganate was used the lamellae appeared as a five or even seven part structure composed of alternate electron dense and electron transparent bands (Echlin, 1964a).

The membranes in the flattened vesicles are usually closely apposed to each other but sometimes the membranes appear to be slightly separated by a narrow intrathylakoidal space (Lang, 1968). The lumen was found to be dilated in aged cells (Echlin, 1964a) and in cells grown under unfavourable conditions, such as nitrogen starvation (Peat and Whitton, 1967; DeVasconcelos and Fay, 1974). Thylakoid irregularity was also found to be associated with aging in A. montana f. minor (Echlin, 1964a), C. fritschii (Peat and Whitton, 1967), Anabaenopsis sp. (Peat and Whitton, 1968) and Scytonema sp. (Whitton, unpublished observations quoted in Lang and Whitton, 1973) and nitrogen starvation in A. cylindrica (DeVasconcelos and Fay, 1974). Under these conditions storage products were found to be accumulated and, according to Lang and Whitton (1973), the presence of numerous storage granules often leads to a much less regular arrangement of the thylakoids. A similar situation was found by Venkataraman, Amelunxen and Lorenzen (1969) when the effect of temperature on the synchronous growth of A. nidulans was determined. Cells harvested from the low temperature phase of the cycle at 26°C

had the individual identity of the thylakoids 'masked' as compared with the 2 or 3 parallel sheets of peripheral thylakoids at the higher temperature of 32°C. It was concluded that the difference was associated with the greater accumulation of photosynthetic products at the lower temperature filling the interlamellar spaces.

Light intensity has been found to affect the thylakoid content of cyanobacteria. For example, Allen (1968a) found an inverse relationship between light intensity and the thylakoid content or pigment concentration in A. nidulans. Also, Peat and Whitton (1967) found that in young filaments of C. fritschii, especially those grown at a low light intensity, the thylakoids were mainly parallel to the cell wall but at higher light intensities, and during later stages of cell development, they became scattered throughout the outer part of the cytoplasm. In cells grown heterotrophically on sucrose in the dark the thylakoids were found to be evenly distributed throughout the cytoplasm. Wildon and Mercer (1963) also showed some reduction in the numbers of lamellae present in dark grown cells.

The effects of carbon dioxide deprivation in S. lividus were investigated by Miller and Holt (1977). A successive loss of thylakoid membranes with an increase in irregularity was found during carbon dioxide starvation, the cells being bleached (containing no chlorophyll a or phycocyanin) after 120 hours of carbon dioxide deprivation and being devoid of detectable thylakoid membrane. It was concluded that the loss of thylakoid membrane, after the exhaustion of pigment and storage materials (carbon and/or nitrogen sources), was the last logical step in the process of utilising cellular reserve materials in times of nutrient shortage. The maintenance of this

intricate membrane system in the absence of available substrate was presumed to be a waste of energy and carbon and thylakoids being composed of lipid and protein could serve as an excellent endogenous source of carbon. The reintroduction of carbon dioxide enriched air into the bleached cultures led to a rapid resynthesis of thylakoid membrane and photosynthetic pigments. A full complement of these components was, however, necessary before growth occurred, indicating that a morphological, chemical and physiological balance of the photosynthetic apparatus had to be attained prior to cell growth.

Certain cyanobacteria produce differentiated cells, akinetes and heterocysts, which also contain thylakoids. According to Lang and Whitton (1973), the arrangement of thylakoids in akinetes is generally similar to vegetative cells prior to enlargement and differentiation but in maturing heterocysts there is a pronounced increase in the amount of photosynthetic membrane.

Apart from the photosynthetic membrane system there have been a few reports of other membranous inclusions in cyanobacteria, for example, lamellasomes in A. nidulans (Echlin, 1964b) and mesosome-like unit-membrane structures in Spirulina and three strains of Synechococcus (Allen, 1972).

1.3.6. Nuclear region

In many cyanobacterial species, particularly the smaller ones, the nuclear material is located in the centre of each cell or along its longitudinal axis (Fuhs, 1973). Echlin (1964a) found that the inner nucleoplasm of A. montana f. minor was an electron dense fibrous-granular material in an electron transparent matrix and

that the outer nucleoplasm seemed to contain ribonucleoprotein particles as described by Ris and Singh (1961). As in bacterial cells, the DNA fibrils are not localised in a membrane-limited nucleus, nor do they ever condense into cytologically demonstrable chromosomes as during mitosis in eukaryotic cells (Holm-Hansen, 1968), nor have histone-type proteins been demonstrated associated with the fibrils (Ris and Singh, 1961; Holm-Hansen, 1968; Lang, 1968; Fogg et al., 1973).

In the best electron microscopy thin sections the nuclear material of cyanobacteria is seen to be composed of DNA fibrils of approximately 2 nm in diameter (Fuhs, 1973). However, different fixation procedures have been shown to affect the degree of aggregation of the finest DNA fibrils (Leak, 1967; Fogg et al. 1973; Fuhs, 1973). Leak (1967) demonstrated three types of fibrils in the nucleoplasm of A. variabilis, by using different fixation methods, ranging in size from 2 - 3 nm in diameter to aggregations from 10 to 35 nm in diameter. A true fixation resulting in an almost homogenous fine-fibrillar appearance of the DNA plasm was obtained using the Kellenberger method (Kellenberger, Ryter and Sechaud, 1958) which is a modified osmium fixation technique, using osmium tetroxide in a solution containing calcium ions and amino acids, followed by uranyl acetate treatment.

Evidence for the location of DNA was provided by the Feulgen test plus autoradiographic techniques following the incorporation of ^{3}H-labelled thymidine into rapidly growing cells of Anabaena sp. (Leak, 1965) and plus UV fluorescence of acridine orange in A. variabilis (Leak, 1967). All of these methods showed that the

nucleoplasmic areas appeared in the central regions of the cells in addition to occupying various regions within the peripheral cytoplasm. These results were substantiated by Leak (1967) who found that cells previously treated with the enzyme desoxyribonuclease or with trichloroacetic acid (both known to extract DNA) did not give the characteristic DNA reactions.

1.3.7. Cytoplasmic inclusions

1.3.7.1. Ribosomes

The most common cytoplasmic elements in cyanobacteria are electron dense granules, 10 - 15 nm in diameter, which can be easily detected by electron microscopy, especially after fixation with osmium tetroxide and staining with uranyl acetate (Ris and Singh, 1961). The ribosomes are especially concentrated around the nucleoplasmic region, in close contact with the DNA fibrils but may also pervade any cytoplasmic space not occupied by other cell components (Ris and Singh, 1961; Lang, 1968; Fogg et al., 1973; Fuhs, 1973). The distribution of RNA was found to correspond to the distribution of these granules as shown by selective removal with the enzyme ribonuclease (Lang, 1968; Fogg et al., 1973). The ribosomes are similar to those found in bacteria, chloroplasts and mitochondria with a Svedburg sedimentation coefficient of 70S, in contrast to the 80S type of ribosome characteristic of the cytoplasm of eukaryotic cells.

1.3.7.2. Cyanophycin (structured) granules

These granules are observed in most cyanobacteria and are located in the periphery of the cytoplasm, having an irregular

spheroidal shape and measuring up to 500 nm in diameter (Fogg et al., 1973; Fuhs, 1973; Wolk, 1973; Shively, 1974). In many filamentous species they are arranged along the cross-walls in a very characteristic manner, sometimes in constant numbers (Lang, 1968; Fuhs, 1973). According to Stewart (1977) these granules stain densely with osmium and often show a striated or punctate appearance under the electron microscope. The identity of cyanophycin granules and structured granules has been confirmed by electron microscope studies on isolated structures (Lang, Simon and Wolk, 1972).

The granules consist of an unusual type of protein with a unique chemical structure containing only two amino acids, arginine and aspartate, in a 1:1 molar ratio forming a highly branched polypeptide (Simon, 1971; Simon and Weathers, 1976). Simon (1973) showed that the formation of cyanophycin granules was enhanced by the presence of a chloramphenicol concentration which completely inhibited protein synthesis so indicating a non-ribosomal system. Consequently, it was suggested that these granules act as cellular nitrogen reserves which form especially when an exogenous nitrogen source is freely available and when protein synthesis is restricted (Simon, 1973; Stanier and Cohen-Bazire, 1977). According to Stanier and Cohen-Bazire (1977) they may also act as an energy reserve as the dissimilation of arginine to ornithine is accompanied by net ATP synthesis.

The number of granules present was found to be lowest in exponentially growing cells and highest in stationary phase cells (Shively, 1974) and were found to develop particularly in nitrogen-rich cultures (Stewart, 1977). Other workers found that these granules were absent in young cells but abundant in older cells and

akinetes (Lang, 1968; Fogg et al., 1973; Wolk, 1973). According to Lang (1968) the association of these granules with resting spores implies a reserve function and their absence or gradual disappearance during akinete germination argues for this premise. The reserve function of these granules was also indicated by their disintegration during nitrogen starvation in A. cylindrica and their abundant formation on addition of ammonia to starved cultures (DeVasconcelos and Fay, 1974). A similar situation occurred due to carbon dioxide starvation in S. lividus (Miller and Holt, 1977), with the cyanophycin granules being lost early in the bleaching process indicating a carbon and/or nitrogen reserve function.

1.3.7.3. Polyglucoside (α or glycogen) granules

These prominent granules are 25 - 30 nm in diameter (Ris and Singh, 1961) and consist of highly branched, high molecular weight polymers of glucose which resemble either glycogen or amylopectin (Shively, 1974). They are generally observed between the thylakoid membranes (Ris and Singh, 1961) and their formation is correlated with active photosynthesis (Lang, 1968; Wolk, 1973). This polymer seems to be the principal non-nitrogenous organic reserve material in cyanobacteria and may be a storage form of energy and/or carbon. The granules react with periodic acid Schiff's reagent for carbohydrate and their selective digestion after diastase and α-amylase incubation also indicates a polyglucoside nature (Lang, 1968; Fogg et al., 1973).

Older cells of T. tenuis and F. diplosiphon were characterised by extensive amounts of carbohydrate storage products (Gantt and

Conti, 1969) and according to Lang (1968) these granules were depleted in developing heterocysts of Anabaena azollae but present in akinetes of Cylindrospermum so showing a correlation with active photosynthesis.

On the other hand, light intensity was found to have no effect on the size, density or distribution of these granules in C. fritschii (Peat and Whitton, 1967), in contrast to the increase in starch grains found in Porphyridium cruentum under high light intensity (Gantt and Conti, 1965). Peat and Whitton (1967) did, however, find a massive accumulation of polyglucoside granules in the peripheral part of C. fritschii cells grown heterotrophically in the dark on sucrose. A similar accumulation of granules was also seen in dark grown cells of N. muscorum supplied with glucose (Wildon and Mercer, 1963).

1.3.7.4. Polyphosphate (volutin or metachromatic) granules

Polyphosphate granules are aggregates of linear polyphosphates of high molecular weight (Fuhs, 1973; Stewart, 1977) forming spherical electron dense bodies 100 - 500 nm in diameter which are generally located in the central area of the cell in close association with the nucleoplasm (Jensen, 1968; 1969; Lang, 1968; Shively, 1974). Polyphosphates are extremely basophilic and so the granules stain readily with any basic dye such as toluidine blue (Fuhs, 1973) or methylene blue, changing its colour to red, hence the name metachromatic (Fogg et al., 1973). They are also acid soluble being extractable with trichloracetic acid or perchloric acid (Harold, 1966; Jensen, 1968). The lead-sulphide staining technique can be used for

electron microscopy identification (Jensen, 1968) although these bodies are not easily preserved by conventional methods of fixation and empty areas in electron micrographs of the cytoplasm generally indicate the removal of polyphosphate due to sectioning (Fogg et al., 1973). The most characteristic behaviour of the granules is seen under intense electron bombardment as the phosphate melts, boils and evaporates in a very characteristic manner leaving a residue resembling a hollow shell (Fuhs, 1973). Phosphate deposition was found to follow a characteristic pattern in P. boryanum grown in continuous illumination in the presence of excess phosphate where the granule was preformed as a porous structure of medium electron density (Jensen, 1969). Polyphosphate was first deposited in the surrounding area of cytoplasm and gradually penetrated into the porous structure until a dense electron opaque body was formed. The presence of other constituents in these granules, such as RNA, DNA, protein and lipid, is still in doubt (Shively, 1974).

The biosynthesis of polyphosphate involves the direct utilisation of ATP although the status of polyphosphate as an energy reserve compound is by no means clearly established (Dawes and Senior, 1973). In fact, the present balance of evidence seems to be against this function and its role as a reserve of phosphorus or a regulator of phosphate economy is favoured.

The deposition of polyphosphate is generally promoted when the growth rate of an organism is low or when its metabolism reflects a nutrient deficiency or imbalance (Harold, 1966; Dawes and Senior, 1973). For example, the granules were small and scarce in young

cultures of A. cylindrica but became large and prominent in old cultures (Fogg et al., 1973). Similarly, the numbers of polyphosphate bodies were high in vegetative cells in lag phase cultures, scarce in rapidly growing log phase cultures and high in post-log phase (that is, stationary) cultures (Stewart, 1977). This phenomenon seemed to be characteristic of a number of different microorganisms, including certain bacteria, yeasts and photosynthetic algae (Harold, 1966; Dawes and Senior, 1973).

Sulphur deficiency was found to lead to a substantial increase in the size and number of polyphosphate granules in cells of A. nidulans (Lawry and Jensen, 1979) as also seen in heterotrophic bacteria (Harold, 1966; Dawes and Senior, 1973). On the other hand, as expected, the granules were found to decrease under phosphate-limiting conditions (Wolk, 1973). The granules also decreased due to extreme carbon dioxide starvation in S. lividus but reformed rapidly when carbon dioxide was introduced into the bleached cultures (Miller and Holt, 1977). Carr and Sandhu (1966) studied the role of polyphosphates in the endogenous metabolism of two photosynthetic microorganisms, the bacterium R. spheroides and the cyanobacterium A. variabilis. It was found that polyphosphate accumulated when the organisms had a source of ATP formation but lacked carbon for growth whereas the deprivation of ATP-forming mechanisms resulted in the degradation of the polymer. For example, polyphosphate in A. variabilis declined on anaerobic incubation in the dark but accumulated on aerobic incubation. However, changes in the polyphosphate content of A. variabilis did not involve more than

20% of the total polyphosphate present so it was concluded that although polyphosphate appeared to act as an energy reserve during endogenous metabolism, this could not be its sole function.

A number of experiments have been carried out on the polyphosphate content of the bacterium A. aerogenes. It was found that under conditions of nutrient imbalance, the accumulation of polyphosphate displayed two separate patterns (Harold, 1966; Dawes and Senior, 1973). One pattern involved the cessation of nucleic acid synthesis due to the exhaustion of an essential metabolite, such as sulphur starvation, and under these conditions a slow deposition of polyphosphate occurred as phosphate was assimilated from the medium. When growth was allowed to resume the accumulated polyphosphate was rapidly degraded and used as a source of phosphorus for the synthesis of nucleic acids and phospholipids. A second pattern was observed when phosphate was added to bacteria previously starved of phosphate and under these conditions a rapid and massive polyphosphate accumulation occurred, designated the 'polyphosphate overplus', which was independent of nucleic acid synthesis and of changes in the composition of the medium. The rate of polyphosphate synthesis was found to be directly proportional to the polyphosphate kinase activity of the organisms (Harold, 1964). However, when growth and nucleic acid synthesis resumed the polyphosphate was degraded and transferred to the nucleic acids.

The antagonistic relationship between polyphosphate and nucleic acid metabolism seems to be a common phenomenon in various organisms (Harold, 1966) and this could be due to the requirement for ATP of polyphosphate kinase, as concurrent nucleic acid synthesis by competing

for available ATP could inhibit the accumulation of the polymer. Evidence of this relationship was also found in A. flos-aquae due to deviations in yield with changing growth rate (Bone, 1971a). It was assumed that phosphate was being converted into the RNA of ribosomes rather than producing reserve material of polyphosphate.

1.3.7.5. Polyhedral bodies (carboxysomes)

Polyhedral bodies have been shown in various autotrophic prokaryotes which fix carbon dioxide via the reductive pentose phosphate cycle (Shively, 1974), including cyanobacteria (Gantt and Conti, 1969; Wolk, 1973; Codd and Stewart, 1976), nitrifying bacteria (Shively, Bock, Westphal and Cannon, 1977) and thiobacilli (Shively, Decker and Greenawalt, 1970; Shively, Ball, Brown and Saunders, 1973a; Shively, Ball and Kline, 1973b; Purohit et al., 1976b). These bodies are large cytoplasmic inclusions with a distinct polygonal profile and granular substructure of medium electron density which are generally located in the nucleoplasmic region of the cell adjacent to the ribosomes and DNA fibrils. Shively et al. (1973b) found that the bodies of T. neapolitanus were bounded by a non-unit membrane 3-4 nm thick. Inclusions isolated from this organism were shown to consist of a mass of 10 nm particles in a paracrystalline array, these particles being identified as RuBPCase, so the name of carboxysomes was proposed (Shively et al., 1973a). These bodies seem to be present in all organisms that can utilise carbon dioxide as the sole carbon source and in thin section they all seem to have

essentially the same appearance. The polyhedral bodies from A. nidulans, for example, showed a membrane-enclosed paracrystalline array of particles (Gantt and Conti, 1969) and the results of a study on A. cylindrica accord with the view that these bodies contain RuBPCase (Codd and Stewart, 1976). According to Shively (1974) it is tempting to propose that these bodies are active in carbon dioxide fixation but present evidence favours an enzyme storage function.

Usually there are several polyhedral bodies present per cell. For example, between 1 and 15 (with 4-6 being most common) bodies were found in T. neapolitanus, the number per cell seemingly directly related to cell size (Shively et al., 1973b). Also, the inclusions seemed to accumulate as the cultures aged (Shively et al., 1970; Purohit et al., 1976b). A similar situation was apparent in A. cylindrica where the bodies were abundant in lag phase cultures, decreased in number during rapid exponential growth and increased again as the growth of the organism slowed in stationary phase (Stewart, 1977).

Purohit et al. (1976b) reported that no RuBPCase nor polyhedral bodies were detected in T. intermedius grown heterotrophically on yeast extract. In contrast, however, Stewart and Codd (1975) found carboxysomes present in many heterocystous and non-heterocystous filamentous cyanobacteria whether they were grown photoautotrophically, photoheterotrophically or chemoheterotrophically so indicating a possible storage function with the enzyme being packed away in a non-active state until needed. In support of the carboxylase function of these bodies, they have been found in akinetes (Stewart

and Codd, 1975) but were consistently absent from heterocysts which do not fix carbon dioxide photosynthetically (Peat and Whitton, 1967; Stewart and Codd, 1975).

Nitrogen starvation was reported to lead to the disintegration of polyhedral bodies in A. cylindrica (DeVasconcelos and Fay, 1974), presumably being broken down for their nitrogen content. In contrast, carbon dioxide-limitation was found to stimulate the formation of carboxysomes in T. neapolitanus (Beudeker et al., 1980). Also, the polyhedral bodies of S. lividus seemed to be the last cytoplasmic bodies to be affected by carbon dioxide starvation (Miller and Holt, 1977) and were the first inclusion bodies to reappear on the addition of carbon dioxide to bleached cultures indicating their importance under these conditions.

1.3.7.6. Phycobilisomes

Phycobilisomes are high molecular weight aggregates of the phycobiliproteins, phycocyanin, allophycocyanin and frequently phycoerythrin (Shively, 1974). According to Wildman and Bowen (1974) they seem to be common to all phycobiliprotein-containing photosynthetic cyanobacteria. They were first detected, however, in the Rhodophytan species P. cruentum (Gantt and Conti, 1965; 1966) and Porphyridium aerugineum (Gantt, Edwards and Conti, 1968). They have since been detected in many cyanobacteria, including T. tenuis and F. diplosiphon (Gantt and Conti, 1969), Gloeocapsa alpicola (Cohen-Bazire and Lefort-Tran, 1970), S. lividus (Edwards and Gantt, 1971; Holt and Edwards, 1972; Golecki, 1979) and A. nidulans, Nostoc sp. and A. quadruplicatum (Gray, Lipschultz

and Gantt, 1973). No such granules were found on the chloroplast lamellae of vascular plants or of any algae not containing phycobiliproteins as accessory pigments (Gantt and Conti, 1966).

Under the electron microscope these inclusions appear as electron dense granules 35-50 nm in diameter which are arranged in regular arrays on the outer (stroma) surface of the thylakoid membranes. They are not integral parts of the thylakoid membranes as they can be washed completely from the thylakoid fragments leaving the chlorophyll and carotenoids in the membranes (Calvin and Lynch, 1952). However, a close spatial association of the phycobiliproteins and the thylakoid chlorophyll is essential for the efficient energy transfer that occurs during photosynthesis (Emerson and Lewis, 1942; Arnold and Oppenheimer, 1950; Duysens, 1951). A structural model for the phycobilisome of P. cruentum has, in fact, been proposed which would allow for maximum energy transfer, a similar arrangement to which would probably also prove to be functional in cyanobacteria (Wildman and Bowen, 1974). The model consists of a core of allophycocyanin in direct contact with the photosynthetic lamella which transfers energy from peripheral phycocyanin and phycoerythrin to chlorophyll a and the reaction centre (Gantt and Lipschultz, 1973; Gantt, Lipschultz and Zilinskas, 1976).

The shape of the phycobilisomes may differ according to species and Gantt et al. (1968) proposed that the shape may be determined by the predominant phycobiliprotein present. P. aerugineum, which contained only phycocyanin, was found to have disc-shaped phycobilisomes whereas P. cruentum, in which phycoerythin predominated over phycocyanin, had spherical-shaped inclusions. A similar result

was obtained for the cyanobacteria A. nidulans which lacked phycoerythrin and Nostoc sp. which possessed all three phycobiliproteins (Gray et al., 1973). On the other hand, Wildman and Bowen (1974) found that the phycobilisomes of a number of cyanobacteria were all discoid in shape irrespective of whether or not they contained phycoerythin and more variations in the sizes of the phycobilisomes were found within individual specimens than could be related to species, age or method of fixation.

However, Evans and Allen (1973) did have problems in the fixation of phycobilisomes in A. nidulans. These bodies were rarely seen in sections of white-light grown cells but were readily seen in red-light grown cells using the same fixation procedure. Under red-light conditions the cells showed a marked reduction in the chlorophyll/phycocyanin ratio owing to a decreased chlorophyll content and it was presumed that preservation was more successful under these conditions as the phycobilisomes were larger. Also, aging was found to affect the phycobilisomes of P. cruentum (Gantt and Conti, 1966), the granules increasing in size as the culture aged. On the other hand, when this organism was grown in nitrogen-deficient media the granules became greatly decreased or absent but reappeared when the nitrogen source was replaced (Gantt and Conti, 1966). A similar situation occurred in S. lividus due to carbon dioxide starvation with the phycobilisomes disappearing in parallel with the phycocyanin content (Miller and Holt, 1977).

1.3.7.7. Other inclusions

Lipid deposits, spherical osmophilic granules of approximately

30-90 nm in diameter, are common cyanobacterial inclusions (Lang, 1968; Peat and Whitton, 1968; Wolk, 1973; Shively, 1974) which are scattered among the thylakoids but more frequently found near the cell surface. These granules are probably reserve products but little is known about their metabolic relations.

Gas vacuoles are present in many cyanobacteria and are a common characteristic of bloom-forming organisms (Fogg et al., 1973; Shively, 1974; Konopka, Brock and Walsby, 1978) and may function in buoyancy provision and regulation, light shielding, surface to volume regulation or a combination of these functions. These inclusions seem to develop most abundantly in cells in dim light but collapse at high light intensities as a result of vigorous photosynthesis (Fogg et al., 1973; Konopka et al., 1978). This means of buoyancy regulation has a definite advantage as the organism can become positioned at a point in the physico-chemical gradient, of light intensity, temperature, nutrient and oxygen concentration, most favourable for its growth (Fogg et al. 1973).

Another type of inclusion, PHB, commonly found in bacteria as a food reserve, was also demonstrated in a cyanobacterium, C. fritschii (Carr, 1966).

1.4. CHEMOSTAT CONTINUOUS-FLOW CULTURE

The continuous culture of microorganisms is a technique of increasing importance in microbiology (Pirt, 1972; 1975; Bull, 1974). Traditionally, batch, or closed, culture systems were used for the study of microbial growth. However, this type of system has no input or output of materials and so is a discontinuous process

in which the environment and the organism are changing continually and therefore only non-steady or transient states can occur. In contrast, continuous-flow, or open, culture systems have a balanced input of growth substances and output of organisms, metabolic products and unused nutrients. Consequently, these systems are capable of attaining steady state conditions in which the average values of all culture and organism properties remain constant and which, in theory, can be maintained indefinitely. The most commonly used continuous-flow culture system is one which is subjected to external control, the growth rate being dependent on the rate of supply of a limiting substrate, and is usually referred to as the chemostat (Monod, 1950; Novick and Szilard, 1950; Herbert, Elsworth and Telling, 1956).

1.4.1. Theory of chemostat continuous-flow culture

1.4.1.1. Kinetics of microbial growth

The growth of a microbial population is an autocatalytic event which, under ideal conditions, proceeds at an exponential rate according to the basic growth equation:

$$\frac{dx}{dt} = \mu x \qquad (1.1.)$$

in which x is the biomass concentration (usually in g) and μ is the specific growth rate which has the units of reciprocal time (h^{-1}). The value of μ is specific for each microorganism and the particular physico-chemical conditions under which it is growing, so it is a function of the genotype and the environment. In the above equation μ is assumed to be a constant but this is

only true when μ is at a maximum value which occurs when all of the substrates necessary for growth are present in excess.

In the case of substrate limitation, the relationship between the concentration of the growth limiting substrate, s, and μ is adequately described by the hyperbolic function developed by Monod (1942):

$$\mu = \mu_{max} \left(\frac{s}{K_s + s}\right) \qquad (1.2.)$$

in which μ_{max} is the maximum specific growth rate which occurs at saturation levels of the substrate and K_s is the substrate saturation constant (numerically equivalent to s at $\mu_{max}/2$ and is characteristic of the organism and the substrate). Determinations of μ_{max} and K_s can be made from double reciprocal plots of the Lineweaver-Burk type by plotting $1/\mu$ against $1/s$. From equation (1.2.) it follows that exponential growth can occur at specific growth rates having any value between zero and μ_{max} provided the substrate concentration can be held constant at an appropriate value.

There is also a simple relationship between growth and the utilisation of substrates. This is shown in its simplest form in growth media containing a single organic substrate and under these conditions the growth rate is a constant fraction, Y, of the substrate utilisation rate:

$$\frac{dx}{dt} = -Y\frac{ds}{dt} \qquad (1.3.)$$

where Y is the observed yield constant being equal to the weight of cells produced divided by the weight of substrate utilised.

1.4.1.2. Dilution rate

In a chemostat continuous-flow culture system, sterile growth

medium is fed into the culture vessel at a steady flow rate (F) and culture emerges from it at the same rate, an overflow device keeping the volume (V) of culture in the vessel constant. A continuous-flow culture is therefore characterised by its fractional rate of medium replacement or its dilution rate (D) which has the units of reciprocal time (h^{-1}):

$$D = \frac{F}{V} \qquad (1.4.)$$

The reciprocal of D is the mean residence time which is defined as the average time an organism remains in the culture vessel. As the doubling time (t_d) becomes longer there is a greater chance that an organism will be washed out of the chemostat before it divides. The washout rate, that is, the rate at which organisms initially present in the culture vessel would be removed if growth ceased but the flow continued, is described by the following equation:

$$-\frac{dx}{dt} = Dx \qquad (1.5.)$$

1.4.1.3. Changes in biomass concentration

In the culture vessel organisms are growing at a rate described by equation (1.1.) and simultaneously being washed away at a rate determined by equation (1.5.) so the net change in x with time is the sum of the growth and output of the cells:

$$\text{increase} = \text{growth} - \text{output}$$

$$\frac{dx}{dt} = \mu x - Dx \qquad (1.6.)$$

Substituting equation (1.2.) for μ:

$$\frac{dx}{dt} = x\left[\mu_{max}\left(\frac{s}{K_s+s}\right) - D\right] \qquad (1.7.)$$

From equation (1.6.) it can be seen that if $\mu > D$, then dx/dt

is positive and the concentration of organisms will increase or if $D>\mu$, then dx/dt is negative and the concentration of organisms will decrease and will eventually washout. However, when $\mu=D$, then $dx/dt = 0$ and x is constant so a steady state culture is obtained with the rate of new biomass production (growth) exactly balanced by the rate of biomass removal (washout).

1.4.1.4. Changes in substrate concentration

A similar balance equation can be formulated for the substrate concentration, s. Assuming that the growth-limiting substrate is entering the culture vessel at a concentration S_R, being consumed by the organisms and flowing out at a concentration s, the net rate of change of substrate concentration is:

increase = input-output-consumption

$$\frac{ds}{dt} = DS_R - Ds - \frac{\mu x}{Y} \qquad (1.8.)$$

Substituting equation (1.2) for μ:

$$\frac{ds}{dt} = D(S_R - s) - \frac{\mu_{max}\, x}{Y}\left(\frac{s}{K_s + s}\right) \qquad (1.9.)$$

From equation (1.8.) it can be seen that if $\mu>D$, then ds/dt is negative and the growth-limiting substrate concentration will decrease or if $D>\mu$, then ds/dt is positive and the growth-limiting substrate concentration will increase. However, when $\mu=D$, then $ds/dt=0$ and the growth-limiting substrate concentration is constant reaching a steady state value at the same time as the biomass concentration.

1.4.1.5. Substrate and organism concentrations under steady state conditions

If S_R and D are held constant and D does not exceed a certain

critical value (designated D_{crit}, see below), then unique values of x and s exist as the system will be in a steady state. Under these conditions dx/dt=0 and ds/dt=0 and the simultaneous equations (1.7.) and (1.9.) can be solved with steady state values of x and s being designated $\bar{x}$ and $\bar{s}$ respectively:

$$\bar{x} = Y(S_R - \bar{s}) \qquad (1.10.)$$

$$\bar{s} = K_s\left(\frac{D}{\mu_{max} - D}\right) \qquad (1.11.)$$

Substituting $\bar{s}$ in equation (1.10.):

$$\bar{x} = Y\left[S_R - K_s\left(\frac{D}{\mu_{max} - D}\right)\right] \qquad (1.12.)$$

From these equations it is possible to predict steady state organism and substrate concentrations in the chemostat for any value of D and concentration of inflowing substrate, provided that the values of the growth constants μ_{max}, K_s and Y are known. It can be seen that the chemostat has a self-adjusting capacity because D determines s (equation 1.11.) which itself determines μ (equation 1.2.) so when $D = \mu$ steady state conditions are restored. This self-adjusting property results in stable growth conditions.

It is clear that D cannot be greater than μ_{max} so there is an upper limit for D, which is nearly equal to μ_{max}, designated D_{crit} (the critical dilution rate). When $D > D_{crit}$ non-steady state conditions are obtained and an exponential washout of the organisms occurs.

1.4.2. Departures from theory

The kinetics discussed above can usually account for the behaviour of chemostat cultures but exact agreement tends to be infrequent as the model is oversimplified and, therefore, must be modified in certain cases. For example, the yield factor (assumed by Monod

to be constant and independent of growth rate) may, in fact, vary with growth rate due to, for example, maintenance energy requirements, change of cell composition or change in efficiency of substrate utilisation. Another variance may result from the effect of population densities due to the excretion of growth stimulatory or growth inhibitory substances. Deviations will also occur if, for example, the growth-limiting substrate is toxic at high D values, if there is imperfect mixing or wall growth in the fermenter or if not all the organisms in a culture are viable.

1.4.3. Advantages of chemostat continuous-flow culture

The chemostat has a number of advantages over other means of culturing microorganisms:

(1) The growth rate can be finely controlled over the growth rate range μ_{min} to D_{crit} (that is, approximately 5-95% μ_{max}) by changing the concentration of the growth-limiting substrate by varying D whilst maintaining a constant environment.

(2) The growth rate can be held constant whilst any physical or chemical parameter is varied systematically, so allowing the effects of these parameters on microbial activity to be determined unequivocally.

(3) Substrate-limited growth conditions can be established with a constant concentration of the limiting substrate. Consequently, steady state conditions of growth can be obtained where the organism is not saturated with substrate as in the case of many batch cultures. Substrate-limitation has wide reaching effects in terms of determining metabolic control and the organism's growth in natural environments.

(4) Organisms can be grown for longer periods under constant conditions. As the biomass of a culture will adjust itself to a steady state in any given environment and due to the constancy of the culture conditions, organisms once growing in a steady state, can be viewed as not having a history. On the other hand, batch culture cells do have a history as the behaviour of organisms at any particular time is influenced by their recent ancestors which have developed under different environmental conditions.

(5) Data obtained from continuous-flow cultures are more reliable and reproducible than those obtained from batch cultures so fewer confirmatory experiments are necessary.

(6) A higher productivity (in terms of biomass) per unit volume per unit time is obtainable, so chemostats are important for large scale biomass production and for the biodegradation of wastes, such as effluents.

1.4.4. Research potential of chemostat continuous-flow culture

The establishment of steady state conditions provides the only unequivocal means of studying phenotypic variation as it enables the experimenter to make precise and systematic analyses of the effects of all environmental parameters on any organism property. Transient state behaviour can also be studied whereby regulatory processes may be revealed and defined when a steady state culture is perturbed by, for example, a stepwise change in dilution rate and adapts to a new steady state condition.

The chemostat continuous-flow technique is now being widely adopted in many areas of microbial research. It has proved

invaluable in investigations on, for example, enzyme regulation (Dean, 1972; Bull, 1974), the macromolecular constitution of organisms as a function of the environment (Herbert, 1961), mutation and selection (Harder, Kuenen and Matin, 1977) and mixed culture studies (Veldkamp and Jannasch, 1972). The chemostat is a superior model for ecological work as it provides high population densities at low substrate concentrations and as such approaches natural populations much more closely.

1.4.5. Light-limited chemostat continuous-flow culture

Light will become the growth-limiting factor when, under certain circumstances at a defined biomass concentration, the average light intensity seen by each cell is less than the saturation light intensity required.

Aiking and Sojka (1979) found that when the dilution rate was maintained at a constant rate and the illumination varied, the situation in a light-limited chemostat was exactly analogous to that in a substrate-limited chemostat in which the concentration of the limiting substrate was varied (Herbert et al., 1956). These workers found that the steady state light-limited culture density of a non-sulphur purple bacterium, R. capsulata, varied directly and linearly with light intensity when maintained at a constant dilution rate. It was concluded that self-shading resulted in all the cells of a light-limited culture at constant D receiving the same actual number of photons irrespective of the light intensity and that this ensured the maximum possible efficiency of utilisation of photons entering the culture chamber.

However, if the light intensity was kept constant and D varied a very different situation was observed from the familiar 'plateau curve' obtained in a substrate-limited chemostat (Herbert et al., 1956). The biomass concentration was found to decrease very rapidly with increasing growth rate, this being due to the 'dilution of light' effect, according to Aiking and Sojka (1979). In a substrate-limited chemostat, if the dilution rate is doubled, for example, then the number of cells being washed out of the vessel per unit time is doubled but so also is the total amount of limiting soluble nutrient entering the vessel per unit time. As a result, the density of the culture will remain nearly constant over a range of dilution rates (the 'plateau'). However, in a light-limited continuous-flow culture device, doubling the dilution rate still doubles the washout rate of cells from the culture but the total number of photons entering the vessel per hour does not increase but remains constant. This results in a reduction of the culture density in a fashion reciprocal with D, and the data can be described by the equation:

$$\bar{x} = \frac{\text{constant}}{D} \qquad (1.13.)$$

A recent study has also been carried out on the effects of light-limitation on a cyanobacterium, Oscillatoria agardhii (van Liere and Mur, 1979). This study was more extensive with factors such as maintenance energy and growth efficiency taken into account but the results obtained were comparable to those above. Again, the biomass concentration was found to decrease rapidly with growth rate. The specific light energy uptake rate (q_E) was found to increase linearly with increasing light intensity and there was a

linear relationship between the specific growth rate (μ) and q_E although, in this case, the slope of the line was dependent on the incident irradiance. The growth efficiency, c (a proportionality factor under a prescribed set of conditions for the conversion of radiant energy into chemical energy) also increased linearly with q_E. Over a wide range of growth rates c was found to be constant but was found to be dependent on the incident irradiance. Also, it was found that μ_e (the specific maintenance rate constant) did not vary with growth rate. The following mathematical expression was derived to explain these results:

$$\mu = c\, q_E - \mu_e \qquad (1.14.)$$

Under constant illumination with changing D, c was constant and, as μ_e is a constant, it can be assumed that under these conditions μ is directly proportional to the specific rate of energy uptake, q_E. However, q_E is also dependent on the incident light intensity (I) and the biomass concentration (x):

$$q_E \propto \frac{I}{x} \qquad (1.15.)$$

Consequently, as $\mu \propto q_E$:

$$\mu \propto \frac{I}{x} \qquad (1.16.)$$

In a chemostat under steady state conditions $\mu = D$, so:

$$D \propto \frac{I}{\bar{x}} \qquad (1.17.)$$

Under constant illumination, I will obviously be constant so that the dilution rate becomes directly proportional to $1/\bar{x}$. Consequently, if D is plotted against $1/\bar{x}$, or $1/D$ against $\bar{x}$,

a straight line relationship should be obtained from the results of a steady state culture.

This relationship is the same as that given by Aiking and Sojka (1979) and was also found in a light-limited culture of a green alga, C. pyrenoidosa (Pipes and Koutsoyannis, 1962). This relationship was also found to hold under CO_2-limited conditions where, in a situation analogous to light-limitation, the limiting nutrient was supplied in the gaseous form at a rate independent of the dilution rate (Pipes, 1962).

On the other hand, Aiking and Sojka (1979) found that they could achieve a situation analogous to the typical 'plateau curve' under light-limited, rather than substrate-limited, conditions when D was varied by compensating for the 'dilution of light' effect. This was carried out by increasing the illumination in parallel with D.

Van Liere and Mur (1979) found an order of magnitude difference in the extrapolated μ_e value between cyanobacteria (0.001-0.004 h^{-1}) and green algae (0.008-0.015 h^{-1}), these differences presumably being correlated with differences in the cellular structure between prokaryotes and eukaryotes. The differences in μ_e and in c (which was generally lower in green algae than cyanobacteria, especially at low light intensities) have strict implications for the growth kinetics of these organisms. At low irradiance, cyanobacteria can exert a higher net growth yield which results in a higher growth rate as compared with green algae, an example being given by their succession with light energy supply as the competing factor (Mur, Gons and van Liere, 1977) (section 1.5.).

1.5. COMPETITION AND SUCCESSION

Microorganisms rarely, if ever, grow as pure cultures in natural environments so growth is likely to be profoundly affected by the presence of other species as well as by the prevailing physical and chemical conditions (Meers, 1971; Fogg et al., 1973; Slater and Bull, 1978). Continuous culture has become important in the study of the complex interrelationships that exist in mixed microbial populations (Meers, 1971; Veldkamp and Jannasch, 1972; Jannasch and Mateles, 1974; Slater and Bull, 1978) as open growth systems, such as the chemostat, simulate the natural environment more closely than closed culture systems with growing populations being continuously supplied with a restricted quantity of a required nutrient and high population densities being obtained at these low substrate concentrations.

The chemostat seems, according to Meers (1971), to be particularly suited to the study of situations in which different organisms compete for a limited supply of an essential nutrient. Competition studies being especially interesting as, according to Slater and Bull (1978), competition is probably the single most important interaction in nature and is the basic mechanism behind natural selection and organism evolution. The outcome of a competitive situation for a single growth-limiting substrate has been found to depend on the affinity of the organisms for the limiting substrate (measured in terms of the saturation constant, K_s) as well as the organisms' growth rates under the given conditions (Veldkamp and Jannasch, 1972; Jannasch and Mateles, 1974; Slater and Bull, 1978). It was predicted that if the saturation curves of two organisms

did not overlap then at any substrate concentration and any dilution rate the slowest growing organism would be selectively excluded. This situation was, in fact, shown under glucose-limiting growth conditions by Jost, Drake, Fredrickson and Tsuchiya (1973) with E. coli displacing Azotobacter vinelandii in all cases due to its higher specific growth rate and lower saturation constant under these conditions. On the other hand, it was predicted that if the saturation curves of both organisms crossed then the result of the competition would be dependent on the dilution rate applied. This situation was shown under lactate-limiting growth conditions by Jannasch (1967) with Spirillum sp. being dominant at low substrate concentrations and low growth rates due to its higher substrate affinity but lower growth constants and Pseudomonas sp. being dominant at high substrate concentrations and high growth rates. A similar case was reported by Meers (1971) who found that under magnesium-limited growth conditions Bacillus subtilis replaced the yeast T. utilis at a dilution rate of 0.08 h^{-1} but that the reverse was true at 0.05 h^{-1} implying that the saturation curves for these organisms under these conditions crossed between the specific growth rates of 0.05 and 0.08 h^{-1}.

In nature many factors, such as light intensity, temperature, pH and extracellular products, may determine the outcome of competition for a growth-limiting substrate. The effects of many such factors on the competition between different organisms, particularly bacteria, have been determined using chemostat continuous-flow techniques. However, according to Fogg et al. (1973) information

obtained from the study of laboratory cultures should be applied only with extreme caution to natural conditions as under these conditions a mixture of species interact with each other in a complex way and there is no ample and steady supply of nutrients or optimum physical conditions.

Temperature has been found to affect the outcome of competition between different species and according to Jannasch and Mateles (1974) an organism which exhibits a rapid physiological response to a certain shift in temperature will have a competitive advantage over a more slowly responding organism. These workers reported the results of competition experiments carried out with a psychrophilic Pseudomonas sp. and a facultatively psychrophilic Spirillum sp. grown at four different temperatures under lactate-limiting growth conditions. At 16°C and -2°C the Spirillum and Pseudomonas species respectively outgrew the other species for the entire range of dilution rates tested. However, at intermediate temperatures selection depended on the concentration of the growth-limiting substrate at which competition occurred with the Spirillum sp. being dominant at low substrate concentrations due to its higher substrate affinity and the Pseudomonas sp. being dominant at high substrate concentrations. Competition among five species of marine phytoplankton was also shown to be highly dependent on temperature although the cell yield at the low growth rates used was relatively independent of this variable (Goldman and Ryther, 1976).

Smith and Kelly (1979) found that thiosulphate-limited competition between the obligate chemolithotroph T. neapolitanus and the

versatile facultative autotroph Thiobacillus A2 was in part a function of pH. In pure culture T. neapolitanus grew faster than Thiobacillus A2 at pH values up to 7.6 but in competition Thiobacillus A2 dominated at pH 7.35 and 7.6. It was concluded that this was possibly due to the excretion of organic compounds into the medium by T. neapolitanus which could be utilised by the other organism. It was also found that coexistence of both organisms occurred under all chemolithotrophic growth conditions tested with the dominant organism comprising 85-99% of the population. This indicated that competition was not the sole interaction between the species as the establishment of seemingly stable mixed populations on a single limiting substrate was not consistent with the theory of competition between two species under such conditions. Competition for a single growth-limiting substrate being expected to result in the selective exclusion of all but one species (Powell, 1958; Veldkamp and Jannasch, 1972; Jost et al., 1973; Jannasch and Mateles, 1974; Taylor and Williams, 1975) with coexistence occurring only if other types of interactions occurred between the species, such as the utilisation of different growth limiting substrates. In the latter case the organisms would occupy different ecological niches as occurs in many natural environments. Smith and Kelly (1979) therefore concluded that the persistence of significant numbers of the other organism in dominant cultures of either T. neapolitanus or Thiobacillus A2 could indicate a complex interaction between the two organisms. This view agrees essentially with the general ecological principle that the more stable a system is, the more complex it is likely to be (Jannasch and Mateles, 1974). A similar situation

was found by Jost et al. (1973) in food web studies in which the predation of a protozoan, Tetrahymena pyriformis, on the two bacterial species, E. coli and A. vinelandii, stabilised the competition between the latter and allowed coexistence in the same habitat.

Another example of coexistence was shown by two species of freshwater diatoms Asterionella formosa and Cyclotella meneghiniana when the growth rate of each species was limited by a different resource (Titman, 1976). On the other hand, competition was found to occur under phosphate-limiting conditions when A. formosa was the competitive dominant as this organism had the lower K_s for phosphate whereas under silicate-limiting conditions C. meneghiniana had the lower K_s and so was dominant. These results, therefore, supported the ecological concept that as many competing species can exist as there are limiting resources. However, it has not been demonstrated that there are enough limiting resources for this mechanism to explain the coexistence of so many species in nature.

In cases of mere competition for a limiting substrate, according to Jannasch and Mateles (1974), the relative initial population size is of no importance (except for the time taken until take-over and displacement is completed), although more complex interactions may result in threshold values for initial cell densities. This situation has, in fact, been shown by Tempest, Dicks and Meers (1967) and Meers and Tempest (1968). It was found that Gram-negative organisms, such as A. aerogenes and Pseudomonas fluorescens, invariably outgrew Gram-positive organisms, such as B. subtilis and B. megaterium, in magnesium-limited chemostat cultures due to a greater affinity for the limiting substrate. However, the ability

of B. subtilis and B. megaterium to outgrow each other or to outgrow T. utilis depended on the inoculum size. This dependence resulted from the presence of a specific extracellular product in the Bacillus spp. cultures which stimulated their growth and uptake of magnesium and depended on the population density.

A similar dependence on inoculum size was shown by Lam and Silvester (1979) for M. aeruginosa which was found to inhibit the growth of Anabaena oscillarioides and Chlorella sp. This inhibitory effect was dependent on high concentrations of initial algal inocula and independent of the initial nutrient concentrations so was assumed to be due to the production of inhibitory extracellular products by this organism. It was concluded that growth inhibitors might be important in controlling species succession and species dominance within the phytoplankton population as,for example, M. aeruginosa is a very common bloom forming species and in succession is one of the most persistent species which might be expected due to its ability to inhibit the growth of other cyanobacteria as well as other groups of organisms, such as the green algae. These workers also showed that A. oscillarioides inhibited the growth of Chlorella sp. although the nature of the inhibition was different. In this case it was concluded that the inhibitory effect was a consequence of nutrient competition with A. oscillarioides competing more effectively for the available phosphate.

Light intensity is another important factor in determining the outcome of a competitive situation. Mur et al. (1977) found that O. agardhii was dominant at low light intensities and dilution rates up to 0.03 h^{-1} whereas Scenedesmus protuberans was dominant

at high light intensities due to its greater specific growth rate. It seems that the growth of cyanobacteria is in general favoured by low light intensities. This is possibly one reason why cyanobacterial blooms do not occur in oligotrophic waters as surface growth is inhibited by the high light intensities. However, in eutrophic waters these organisms can grow in layers near the surface due to the decreased light intensities which occur under these conditions. Also, in eutrophic systems, an increase in the concentration of a nutrient, such as phosphate, will lead to an increase in plankton biomass and the resulting increase in mutual shading will then cause a diminishing average light intensity so favouring the growth of a number of cyanobacterial species over the growth of eukaryotic algal species.

The rate of carbon dioxide supply is also an important factor in regulating the qualitative nature of the phytoplankton (Shapiro, 1973). Studies were carried out in a Minnesota Lake and it was found that the addition of carbon dioxide or lowering the pH stimulated a shift of dominance from cyanobacteria to green algae especially when nutrients were supplied simultaneously, whereas just an addition of nutrients led to an increased cyanobacterial population. It seemed that cyanobacteria were more efficient at obtaining carbon dioxide from low concentrations than green algae and it was predicted that under circumstances when the pH was high, such as in enriched lakes, cyanobacteria should predominate. On the other hand, the addition of free carbon dioxide or the lowering of the pH made carbon dioxide more available and the

presence of nutrients allowed the green algae to take advantage of this availability. It was thought that these results would explain the closely analogous situation that occurs when a stratified lake is mixed. In this situation nitrogen, phosphorus and carbon dioxide are known to be brought up from the hypolimnion to the epilimnion and carbon dioxide enters from the atmosphere. In consequence mixing usually results in a lowered pH and, therefore, the kinetics of nutrient uptake and use by green algae allows these organisms to dominate the cyanobacteria.

Even though it is difficult to correlate laboratory work directly with the natural situation many of these factors, such as temperature, light intensity, nutrient uptake, extracellular metabolites and carbon dioxide availability, have been implicated in affecting the succession of cyanobacteria in natural systems.

Cyanobacteria are scarcely ever absent from the plankton although these organisms tend to be most abundant in the summer and autumn (Fogg et al., 1973; Konopka and Brock, 1978a; 1978b), their growth being favoured by low light intensities and high water temperatures. Casterlin and Reynolds (1977) found that in a eutrophic North Temperate lake in Pennsylvania diatoms were dominant in the winter and spring, Chlorophyta in the summer and autumn and cyanobacteria reached a maximum in late summer but were also abundant in autumn and winter. It was concluded that the selective advantage of heterotrophic and photoheterotrophic assimilation of organic substrates, which is common among diatom species, under low light intensities, could partly explain the diatom dominance in winter and early spring whereas a combination of factors, such as increased

light intensity and water temperature, reduced circulation and silica depletion, was possibly responsible for the succession of spring diatoms to summer Chlorophyta. It was thought that cyanobacteria might dominate in late summer due to the higher water temperatures and their competitive advantage when nutrient concentrations were depleted. Interaction between algal species was also implicated as being involved in seasonal succession, such as the release of toxic substances or decomposition which could lead to nutrient regeneration and further enrichment of the water for subsequent populations. The release of metabolic products by cyanobacteria has, in fact, been shown to play a significant role in determining the annual bloom sequence in a eutrophic freshwater lake, Linsley Pond, in Connecticut (Keating, 1977; 1978).

According to Fogg et al. (1973) the growth of planktonic cyanobacteria seems to be favoured by dissolved organic matter although this correlation does not seem to depend on any simple direct mechanism as no freshwater cyanobacterial species have been found which require exogenous organic growth factors. It was suggested that this may be due to the decreased light intensity under these conditions or due to bacteria growing on the organic matter and producing carbon dioxide which can be utilised by the cyanobacteria, although it seems that carbon dioxide is rarely severely limiting under lake conditions. On the other hand, oxygen depletion is likely to be greater when the concentration of dissolved organic matter is high and this would favour cyanobacterial growth as metabolic functions, such as nitrogen fixation and photosynthetic carbon dioxide fixation,

are inhibited by high levels of oxygen and cyanobacteria tend to grow more rapidly under microaerophilic than fully aerobic conditions.

Also according to Fogg et al. (1973) cyanobacterial growth seems to be favoured by low nutrient concentrations. In the case of planktonic cyanobacteria which fix nitrogen good growth would be expected to occur in the absence of combined nitrogen but species which apparently do not fix nitrogen, such as M. aeruginosa, may be as abundant as nitrogen-fixing forms at times of nitrate deficiency. It seems that the organisms may store previously available nitrogen in the form of cyanophycin granules (section 1.3.7.2.) or phycocyanin (section 1.2.3.) which they can utilise under nitrogen-limiting conditions. Similarly, growth in waters low in phosphorus may be due to organisms utilising phosphorus which they had previously accumulated as polyphosphate (section 1.3.7.4.) or may be due to phosphate uptake kinetics which tend to favour cyanobacteria rather than green algae (Lam and Silvester, 1979). An indication that cyanophycin granules and polyphosphate bodies acted as stores of nitrogen and phosphorus respectively was given by Stewart (1977) who found that at the height of bloom formation of Microcystis flos-aquae in Balgavies Loch, near Dundee, the organisms had become depleted of these bodies even though polyhedral bodies were still present. It seems paradoxical that although they tend to develop at times of nutrient deficiency, planktonic cyanobacteria are characteristic of waters receiving high nutrient inputs (Fogg et al., 1973). The situation is obviously very complex with many factors being involved.

DeNoyelles and O'Brien (1978) found that nitrogen and phosphorus

enrichment of oligotrophic experimental ponds selectively stimulated the growth of a number of originally rare eutrophic species, mostly members of the Chlorophyta and Cyanobacteria. It was found that phytoplankton changes in terms of overall biomass was directly associated with the level of nitrogen and phosphorus enrichment and in some cases also with zooplankton grazing, and that the species composition was not only directly related to the level of nutrient addition but also to associated conditions, including changes in inorganic carbon availability. During the summer, the midday pH rose to 10 or above due to the increased plant productivity and free carbon dioxide declined so causing an advantage to species still capable of active free carbon-dioxide uptake at these very low concentrations, such as cyanobacteria, or having the ability to use bicarbonate as a carbon source. It was also suggested that cyanobacteria may possess other advantages, such as grazing resistance, increased buoyancy and the ability to fix nitrogen. The ability of buoyancy regulation, due to the possession of gas vacuoles (section 1.3.7.7.), is a definite competitive advantage to many bloom forming cyanobacteria as, for example, they can maintain an optimum position in the water and can migrate rapidly to the surface layers (that is, the eutrophic zone) during calm periods after turbulent mixing (Fogg et al., 1973; Konopka et al., 1978).

The ability of nitrogen fixation may also be a determining factor in succession. For example, according to Fogg et al. (1973), a diatom Fragilana crotonensis developed first in Linsley Pond, Connecticut, when the summer nitrate-nitrogen concentration was slightly over 0.7 mg l^{-1} and was succeeded by Anabaena circinalis.

a nitrogen fixing cyanobacterium, when this concentration had fallen to approximately 0.1 mg l^{-1}. In the following year when the nitrate-nitrogen concentration was lower, 0.3 mg l^{-1}, the A. circinalis maximum preceded that of F. crotonensis. Walmsley and Ashton (1977) also found that the availability of combined nitrogen seemed to play a major role in governing the succession of algae in Rietvlei Dam, South Africa, although environmental factors, such as water temperature and inflow, were important as well. It was shown, for example, that a bloom of the green alga Volvox rousseletii utilised almost all of the available combined nitrogen but relatively large quantities of available phosphate were still present so this led to a succeeding bloom of the nitrogen fixer A. circinalis. This was followed, due to increased levels of combined nitrogen, by a bloom of the non-nitrogen fixing cyanobacterium M. aeruginosa.

1.6. AIMS OF WORK

The aim of this investigation was to determine the effects of various environmental conditions on the growth, physiology and ultrastructure of the cyanobacterium A. nidulans grown under chemostat continuous-flow culture conditions.

The influence of growth (dilution) rate and light-and carbon dioxide-limitation on the basic growth characteristics, macromolecular composition, in terms of DNA, RNA, protein, pigment, carbohydrate and lipid, ultrastructure and activity of the carboxylase enzymes RuBPCase, PEPCase and pyruvate Case of this organism were studied. The effects of competition on A. nidulans of a green alga, Scenedesmus quadricauda, were also determined at different growth rates under light-and carbon dioxide-limiting growth conditions.

PART 2. MATERIALS AND METHODS

2.1. ORGANISMS

The cyanobacterium, Anacystis nidulans used in these studies was kindly supplied by Dr. N. G. Carr, University of Liverpool. This organism is known as A. nidulans strain 6301 according to Stanier, Kunisawa, Mandel and Cohen-Bazire (1971) or as Synechococcus PCC 6301 according to Herdman, Janvier, Rippka and Stanier (1979), the original A. nidulans strain being number 625 from the Indiana University Culture Collection.

The green alga, Scenedesmus quadricauda, used in the competition studies (section 2.10) was obtained from The Culture Centre of Algae and Protozoa, Cambridge.

2.2. CULTURE MAINTENANCE

2.2.1. Maintenance in liquid culture

2.2.1.1. A. nidulans

Liquid stock cultures of A. nidulans were maintained in closed culture using Medium C (Kratz and Myers, 1955) as modified by Slater (1975) supplemented with 0.5 g l^{-1} $NaHCO_3$. The growth medium contained, in g l^{-1} glass-distilled water: KNO_3, 1.0; K_2HPO_4, 1.0; $Ca(NO_3)_2$, 0.025 and $MgSO_4.7H_2O$, 0.25; plus 1.0 ml of Arnon's A5 trace element solution (Allen and Arnon, 1955) containing, in g l^{-1} glass-distilled water: $MnCl_2.4H_2O$, 1.81; $ZnSO_4.7H_2O$, 0.222; $CuSO_4.5H_2O$, 0.079; $Na_2MoO_4.2H_2O$, 0.0252 and H_3BO_3, 2.86; and 1.0 ml FeEDTA solution containing, in g l^{-1} glass-distilled water: NaEDTA, 6.34 and $FeSO_4.7H_2O$, 4.98.

Flasks (100 ml) containing approximately 20 ml Medium C plus $NaHCO_3$ were autoclaved at 10 lbf in $^{-2}$ for 10 minutes. The cultures were incubated at 25°C and illuminated by six 8W White fluorescent tubes placed between 3 and 15 cm from the flasks in an illuminated cooled incubator (Gallenkamp, London) and were subcultured every 15 - 20 days.

2.2.1.2. S. quadricauda

Liquid stock cultures of S. quadricauda were maintained in closed culture using a green algal medium modified from that used by John and Syrett (1967). The growth medium (GAM) contained, in g l^{-1} glass-distilled water: KH_2PO_4, 0.7; K_2HPO_4, 0.3; $MgSO_4.7H_2O$, 0.3; urea, 1.0 and thiamine, 1 x 10^{-8}; plus 1.0 ml of Arnon's A5 trace element solution (section 2.2.1.1.) and 1.0 ml FeEDTA solution (section 2.2.1.1.).

Autoclaving and culturing conditions were the same as for A. nidulans (section 2.2.1.1.).

2.2.2. Maintenance on defined solid medium

2.2.2.1. A. nidulans

A. nidulans was also maintained on agar slopes in Pyrex-glass test tubes fitted with polypropylene caps (C. I. Clark and Co., Hampton, Middlesex) and on agar plates. These were subcultured frequently, usually every 15 days, to maintain an axenic culture. Cyanobacteria in general take a long time to grow on solid media although it was found that reasonable growth could be obtained after 2 - 3 days using the following procedure.

The same quantity of mineral medium (Medium C) supplemented with 0.5 g $NaHCO_3$ l^{-1} and 1.5% (w/v) agar ('lab m') both at double strength were sterilised separately by autoclaving at 15 lbf in^{-2} for 20 min, combined after cooling to approximately 45°C and the Petri dishes and slopes were poured. These media were left 2-4 days before inoculation and were incubated at 25°C in the light as described before (section 2.2.1.1.).

2.2.2.2. S. quadricauda

S. quadricauda was maintained on solid media prepared from GAM using the preparation procedure as above (section 2.2.2.1.). These media were left 2-4 days before inoculation and were incubated at 25°C in the light as described before (section 2.2.1.1.). These were subcultured approximately every 15 days.

2.3. PURIFICATION OF THE CULTURES

All cultures were checked at regular intervals for heterotrophic bacterial contamination by plating out onto nutrient agar. The agar was used at the recommended concentration of 28 g l^{-1} and autoclaved at 15 lbf in^{-2} for 20 min. The Petri dishes were incubated as described previously (section 2.2.2.1.). Pure cultures were obtained from contaminated ones by continual restreaking of single colonies onto mineral media agar plates (sections 2.2.2.1. and 2.2.2.2.) to remove the contaminating bacteria (Stanier et al., 1971).

2.4. AUTOTROPHIC CHEMOSTAT CONTINUOUS-FLOW CULTURE

2.4.1. Growth media

2.4.1.1. Light-limited medium

The growth medium used under these conditions was the modified Medium C (section 2.2.1.1.). The fresh medium reservoir (20 l Pyrex glass bottle) was filled with 17 l Medium C and autoclaved at 15 lbf in^{-2} for 40 min.

2.4.1.2. Carbon dioxide-limited medium

The growth medium used under these conditions was the modified Medium C (section 2.2.1.1.) supplemented with $NaHCO_3$ to give a final concentration of 0.42 g l^{-1} (5mM). The $NaHCO_3$ was sterilized separately from the basal medium and added after cooling. The $NaHCO_3$ was autoclaved in 25 ml glass Universal bottles (for 17 l medium, 7.25 g $NaHCO_3$ was dissolved in 150 ml distilled water) at 10 lbf in^{-2} for 10 min and added to the basal Medium C in the reservoir which had previously been autoclaved at 15 lbf in^{-2} for 40 min and cooled. This concentration of bicarbonate was used since Karagouni (1979) found that the culture biomass was directly proportional to the bicarbonate concentration between 2 and 7.5 mM. Thus, under these growth conditions, bicarbonate was the only limiting nutrient. 5 mM $NaHCO_3$ was used in all experiments as this gave an absorbance of approximately 0.5 at 600 nm and the expected substrate-limited pattern of growth.

2.4.2. Light-limited continuous-flow culture system

The basic features of the light-limited continuous-flow culture system used to provide the controlled autotrophic growth

of A. nidulans are shown in figure 2.1.

The growth vessel (G) was a 2.5 l Quickfit Pyrex glass vessel (L. H. Engineering, Stoke Poges) with a working culture volume of 2.0 l. This was fitted with a glass lid (L) containing five inlet and outlet ports for media and gas supply and removal, sampling, the cooling finger and the thermometer pocket.

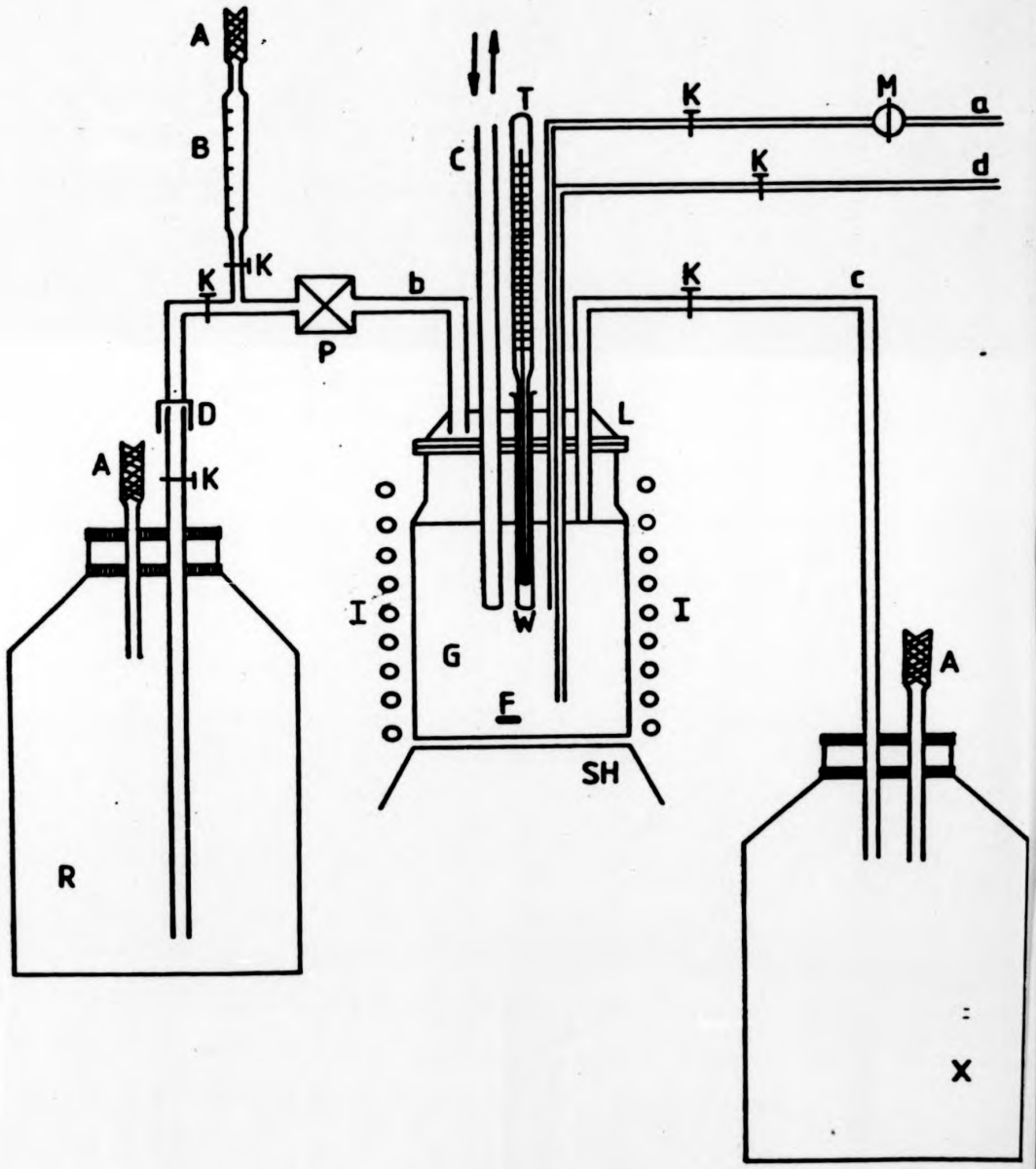
For growth within the vessel, the culture was continuously sparged via the gas inlet (a) with 5% CO_2:95% N_2 (v/v) sterilized by passage through an autoclavable membrane filter (M) (Microflow Limited, Fleet, Hants). The temperature within the vessel was controlled to 40°C with an accuracy of approximately ±0.5°C, optimum growth being obtained at this temperature (Karagouni, 1979), by a combination of a cooling finger (C) and the heater element of the combined magnetic stirrer/heater (SH) (S 'mag' H, Voss Instruments Limited, Maldon). Tap water was passed continually through the cooling finger at a low flow rate to control the temperature of the culture. The culture temperature was measured by a thermometer/thermostat unit (the Electrical Thermometer Company Limited, Thetford) the thermometer (T) being situated centrally in the culture vessel lid in a Pyrex glass pocket (W) filled with distilled water. The thermostat was connected via an electronic switching relay (Gallenkamp, London) to the heater unit of the magnetic stirrer for temperature regulation.

The culture was stirred continually by means of a 5 cm magnetic follower (F) operated by the magnetic stirrer/heater (SH).

Figure 2.1.

A schematic representation of the light-limited chemostat.

A, air glass filters; B, burette; C, cooling finger; D, connecting hood; F, magnetic follower; G, growth vessel; I, growth lights; K, Hoffman Clips; L, culture vessel lid; M, membrane filter; P, flow inducer; R, fresh medium reservoir; SH, magnetic stirrer/heater; T, thermometer; W, water pocket; X, waste culture reservoir. a, gas inlet; b, fresh medium inlet; c, growth vessel overflow; d, sampling tube.


A
B
K
K
P
b
D
A
K
R
T
C
K
M
a
K
d
K
c
L
I
I
G
W
F
SH
A
X

The rate of stirring was kept sufficiently rapid to ensure that a homogeneous culture was maintained and to minimise wall growth. The culture was illuminated on two sides by nine 8 W Warm White fluorescent tubes (I) placed 10 cm away from the culture vessel. The light tubes were replaced regularly to ensure that the quality of the light source and the intensity remained constant. The light intensity in the centre of the culture was difficult to determine because of the shape and volume of the culture vessel but it was in the order of 10,000 lux.

A 20 l Pyrex glass vessel (R) was used as the fresh medium reservoir and sterile fresh medium (section 2.4.1.1.) was pumped from this reservoir into the growth vessel through an inlet port (b) by means of a peristaltic flow inducer (P) (MHRE 7, Watson-Marlow Limited, Falmouth, Cornwall). There was a connecting hood (D) on the feedline between the reservoir and flow inducer which could be disconnected to allow separate autoclaving of the reservoir and the rest of the chemostat. A burette (B) was also placed in the medium input line for the determination of the flow rate of the fresh medium. The time taken to transfer a known volume of medium from the burette to the culture vessel was determined and the flow rate calculated in terms of ml h^{-1}. From this the dilution rate D (h^{-1}) was calculated (section 1.4.1.2.).

Waste culture and effluent gases were removed from the culture vessel through the gas/waste outlet port (c) into the waste culture reservoir (X). During continuous-flow culture

fresh medium was added continuously to the culture vessel and as the volume increased the end of the effluent tube became covered. The culture vessel was continually gassed so this resulted in a temporary increase in pressure with the consequent removal of an aliquot of culture and the waste gases were permitted to escape. The waste culture vessel was a 20 l Pyrex glass vessel and when full was removed aseptically and substituted by an empty sterile waste bottle.

The continuous-flow culture system was autoclaved as two separate units, the fresh medium reservoir being disconnected from the rest of the chemostat at the connecting hood. The fresh medium reservoir was autoclaved as described previously (section 2.4.1.1.) and the rest of the chemostat was autoclaved empty as a single unit at 15 lbf in^{-2} for 20 min. After sterilisation the growth vessel was positioned on the magnetic stirrer between the lights and the fresh medium line was aseptically connected to the fresh medium reservoir. The culture vessel was filled with fresh medium ready for inoculation.

2.4.3. Carbon dioxide-limited continuous-flow culture system

The basic features of the carbon dioxide-limited continuous-flow culture system used to provide the controlled autotrophic growth of *A. nidulans* are shown in figure 2.2.

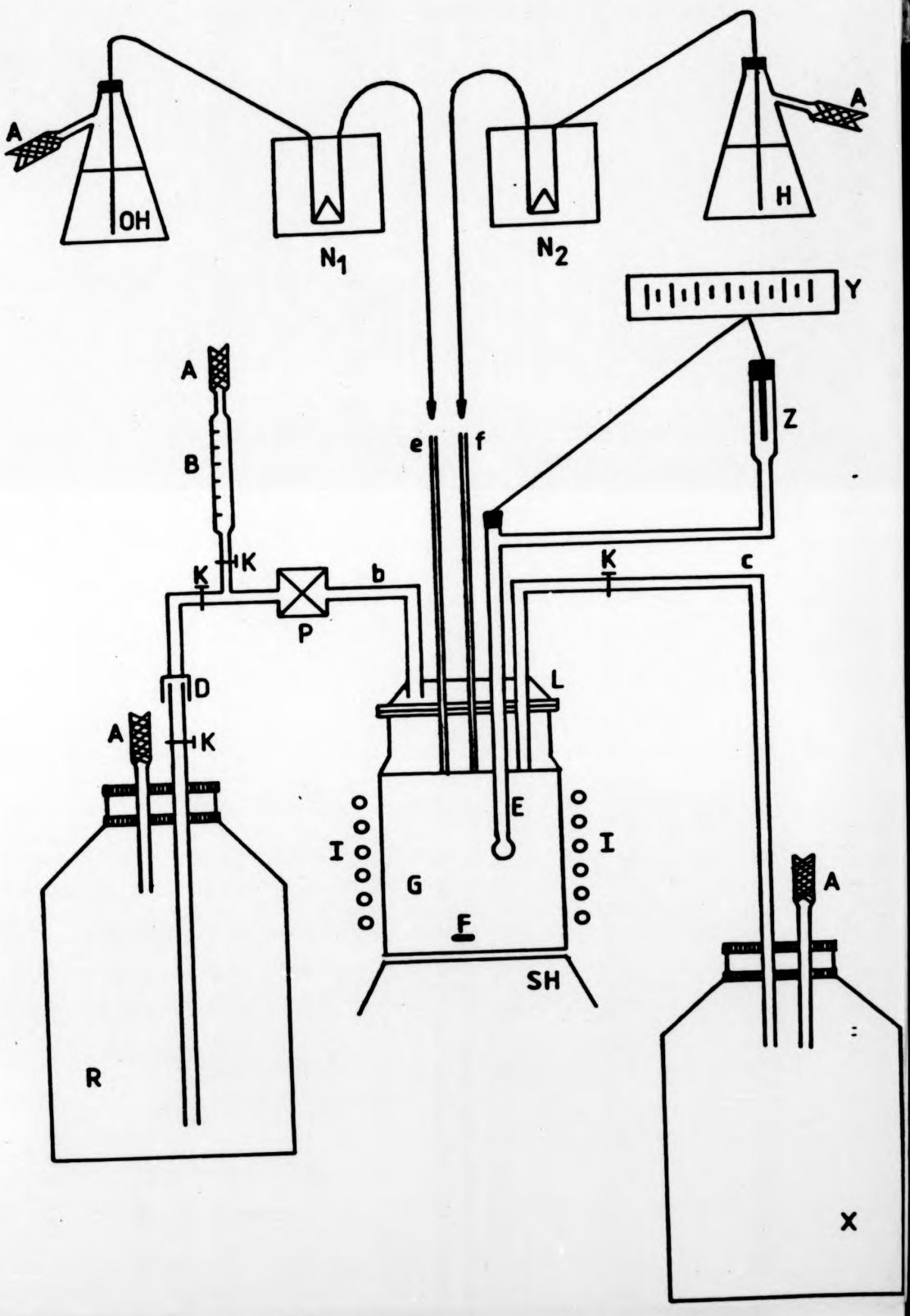
The basic system was similar to the light-limited chemostat except for the addition of pH monitoring and controlling facilities (EIL Model 9150 pH controller, Chertsey, Surrey). The pH controller (Y) was used to monitor the pH within the culture vessel

Figure 2.2.

A schematic representation of the carbon dioxide-limited chemostat.

A, air glass filters; B, burette; D, connecting hood; E, glass electrode; F, magnetic follower; G, growth vessel; I, growth lights; K, Hoffman clips; L, culture vessel lid; N_1 and N_2, Shuco triangular flow-inducers; OH, flask containing 2N NaOH; H, flask containing 2N HCl; P, flow inducer; R, fresh medium reservoir; SH, magnetic stirrer/heater; X, waste culture reservoir; Y, pH controller; Z, reference electrode. b, fresh medium inlet; c, growth vessel overflow; e, NaOH inlet; f, HCl inlet.

The cooling finger, thermometer and its water pocket, gas inlet and sampling port are not represented in this figure.


A
OH
N1
N2
A
H
Y
Z
A
B
K
K
b
P
e
f
K
c
D
A
K
L
E
I
I
G
F
SH
R
A
X

and maintain a value of pH 7.5 ± 0.2 which was the optimum pH for the growth of this organism (Karagouni, 1979). This system was required because the addition of the $NaHCO_3$ solution to the fresh medium caused the pH to increase to a value between pH 9 and 10 and under these conditions A. nidulans could not grow. The pH controller was connected to a glass electrode (E) with a remote calomel reference electrode (Z) containing 3.8M sterile KCl and to two Shuco triangular flow inducers (N_1 and N_2) (Saia, London) which added appropriate volumes of either 2N NaOH or 2N HCl to the culture vessel (via inlet ports e and f respectively) for pH correction.

The culture was continuously sparged with N_2 gas through an autoclavable membrane filter and was illuminated on two sides by five 8 W Warm White fluorescent tubes and one 8 W Gro-lux fluorescent tube placed 10 cm from the culture vessel (I). In addition, two 100 W tungsten filament light bulbs were placed 15 cm behind the culture vessel.

The continuous-flow culture system was autoclaved as two separate units as described for the light-limited system (section 2.4.2.). The pH glass electrode could not be autoclaved and so was sterilised in 70% (v/v) absolute alcohol for 25 to 30 min and washed with approximately 500 ml sterile glass-distilled water before being placed aseptically into the sterile growth vessel.

2.4.4. Inoculation

2.4.4.1. Light-limited system

The growth vessel was inoculated aseptically through one of

the inlet ports with a few colonies of A. nidulans taken from solid media stock cultures (section 2.2.2.1.). The organisms were initially grown as a batch culture without stirring. This allowed the culture to settle at the bottom of the growth vessel and was important in promoting initial growth. Once growth had been established the culture was stirred continuously and when an absorbance of approximately 1.0 was attained the continuous flow of fresh medium was started.

2.4.4.2. Carbon dioxide-limited system

It was more difficult to establish cultures under carbon dioxide-limited conditions than under light-limited conditions. In this case it was necessary to add at least 100 ml of an exponentially growing inoculum to the growth vessel. The culture could not be left in batch growth conditions for longer than a few hours due to the low carbon dioxide concentration and so the flow of fresh medium was started almost immediately. The flow rate was initially low, giving a dilution rate of approximately 0.05 h^{-1}, in order to prevent washout of the culture but was increased after growth had been established.

2.4.5. Sampling

The Hoffman clip (K) on the sample tube (d) was opened and at the same time the Hoffman clip (K) on the waste medium line (c) was closed to prevent the sparging gas from leaving the growth vessel. This caused a rise in pressure within the culture vessel which forced the culture down the sample line and this

was collected in a 25 ml glass universal bottle with a screw top.

2.5. DETERMINATIONS OF BASIC GROWTH CHARACTERISTICS FOR A. NIDULANS UNDER CONTINUOUS-FLOW CULTURE CONDITIONS

2.5.1. Determination of a steady state

To obtain a steady state culture the dilution rate (D) was kept constant for at least three times the culture doubling time (t_d). After this time the culture absorbance (section 2.5.2.) was measured at least twice a day and if the absorbance remained constant a steady state was established. All determinations under continuous-flow conditions were carried out on steady state cultures.

2.5.2. Determination of culture absorbance

The culture absorbance was determined at 600 and 650 nm in a Unicam SP 1700 Spectrophotometer using 3.0 ml glass cuvettes with a 1 cm light path.

2.5.3. Estimation of culture biomass

The culture biomass was determined by filtering a known volume of culture through a predried and preweighed 0.45 µ pore size HAWP 22 mm diameter Millipore filter and drying to constant weight at 100°C for 18 hours. The results were expressed as mg dry weight ml^{-1} and as pg dry weight $cell^{-1}$.

2.5.4. Estimation of cell number

Samples for cell number determination were fixed in an equal

volume of 2.5% (v/v) glutaraldehyde solution. These samples were stored at 4°C until counts could be carried out. The cell number was determined in a suitably diluted cell suspension in a Neubauer counting chamber (Gallenkamp, London). The values given were the mean of three separate determinations and the results were expressed as numbers of organisms ml^{-1}.

2.6. MACROMOLECULAR ANALYSES

2.6.1. Nucleic acid determination

2.6.1.1. DNA determination

Steady state cultures were harvested by centrifugation at 15,000 x g for 15 min and the pellet resuspended in distilled water to give a final concentration of approximately 1×10^9 organisms ml^{-1}. Eight samples, each containing between 2 to 8×10^9 organisms, were taken and cooled on ice. 60% (v/v) perchloric acid was added to give a final concentration of 5% (v/v) and the samples left to stand on ice for 15 min. The samples were centrifuged (15,000 x g for 15 min), the supernatant discarded and the pellets resuspended in an equal volume of 2.5% (v/v) ice-cold perchloric acid. After standing on ice for a further 15 min, the samples were centrifuged, the supernatant discarded and the pellet resuspended in 2.0 ml 5% (v/v) perchloric acid and incubated at 70°C for 40 min. The samples were cooled on ice, centrifuged and 1.0 ml samples of the supernatant assayed for DNA by the diphenylamine method of Burton (1956).

2.0 ml freshly prepared diphenylamine reagent (consisting of 1.5% (w/v) diphenylamine in glacial acetic acid, 1.5% (v/v) concentrated H_2SO_4 and 0.5 ml 1.6% (v/v) acetaldehyde) was added to 1.0 ml supernatant or to 1.0 ml 5% (v/v) perchloric

acid for the blank. These samples were incubated at room temperature (20 - 30°C) in the dark for 17 - 20 hours and the absorbance of the samples was determined at 600 nm against the blank.

The values were compared with 2-deoxy-D-ribose standards and the DNA estimated on the basis of 1 µg 2-deoxy-D-ribose being equivalent to 5.6 µg DNA. The DNA contents were expressed as fg DNA $(cell)^{-1}$ and as a percentage of the cell dry weight. For each steady state culture at a known dilution rate and a steady state biomass concentration, the specific rate of DNA formation, q_{DNA}, was calculated in terms of ag DNA $(g\ dry\ weight)^{-1}h^{-1}$. This was derived from the following generalised equation of Pirt (1975) for the rate of product formation under steady state conditions in chemostat culture:

$$q_p = \frac{\bar{p}D}{\bar{x}}$$

where q_p is the specific rate of product formation and $\bar{p}$ and $\bar{x}$ are the steady state product and biomass concentrations respectively.

2.6.1.2. RNA determination

Steady state cultures were harvested by centrifugation at 15,000x g for 15 min and the pellet resuspended in distilled water to give a final concentration of approximately 1×10^9 organisms ml^{-1}. Eight samples, each containing between 0.5 to 2.0 x 10^9 organisms were cooled on ice, centrifuged and the supernatant discarded. The resulting pellets were washed twice for 10 min in absolute ethanol and the organisms resuspended in

2.5 ml 0.2 M NaCl and 0.2 ml 60% (v/v) perchloric acid and incubated at 70°C for 40 min. The samples were cooled on ice, centrifuged and 1.0 ml samples of the supernatant assayed for RNA by a modification of the orcinol reaction (Schneider, 1957) stated by Mann and Carr (1974).

1.0 ml 0.05% (w/v) $FeCl_3$ in concentrated HCl and 0.1 ml 6.0% (w/v) orcinol (5-methyl resorcinol) in ethanol were added to 1.0 ml supernatant or to 1.0 ml distilled water for the blank. These samples were incubated at 100°C for 30 min and cooled on ice. The colour was stabilised after cooling by the addition of 1.0 ml 20% (v/v) ethanol. The absorbance of the samples was determined at 650 nm against the blank.

The values were compared with D(-)ribose standards and the RNA estimated on the basis of 1 µg ribose being equivalent to 4.3 µg RNA. The RNA contents were expressed as fg RNA $cell^{-1}$ and as a percentage of the cell dry weight. The specific rate of RNA formation, q_{RNA}, was calculated in terms of mg RNA (g dry weight)$^{-1}$ h^{-1} in the same way as the q_{DNA} (section 2.6.1.1.).

2.6.2. Protein determination

The protein content of a suitably diluted washed cell suspension from a steady state culture was determined by the method of Lowry, Rosebrough, Farr and Randall (1951).

A series of assay tubes containing 0.1 to 0.4 ml of the cell suspension and made up if necessary to 0.4 ml with distilled water were set up as well as a distilled water blank. 2.0 ml fresh solution C (50 ml solution A, 2% (w/v) sodium carbonate

in 0.1 N sodium hydroxide, 0.5 ml solution B_1, 1% (w/v) copper sulphate and 0.5 ml solution B_2, 2% (w/v) sodium potassium tartrate) was added to each 0.4 ml sample. This was mixed and left to stand at room temperature for 10 min. 0.2 ml solution D (Folin and Ciocalteu's phenol reagent diluted 1.0 ml with 1.5 ml distilled water) was added and mixed thoroughly and rapidly. After 30 min the absorbance was determined at 700 nm against the blank.

A standard curve was prepared using bovine serum albumin at concentrations of 5-200 µg and from this the protein content of the organisms was estimated.

It was considered that the pigmentation of the cells might interfere with this spectrophotometric assay method so the method of Vernon and Kamen (1953) was used to remove the pigments by three extractions with boiling 80% (v/v) ethanol acidified with acetic acid. The precipitated protein was diluted with water and the protein content determined on an aliquot using the Lowry method as before.

The protein contents were expressed as pg protein $cell^{-1}$ and as a percentage of the cell dry weight.

2.6.3. Pigment determination

2.6.3.1. Chlorophyll a and total carotenoid determination

Steady state cultures were harvested, washed and resuspended in a known volume of distilled water to give an absorbance reading of approximately 1.5 at 600 nm.

A known volume of the suspension was taken, centrifuged and the pellet extracted twice with 80% (v/v) acetone for 5 min. The supernatants were combined and the absorption spectrum determined on a Unicam SP 1700 spectrophotometer using an 80% (v/v) acetone blank in 1.0 ml 1 cm pathlength cuvettes. The chlorophyll a and total carotenoid contents of the cells were determined from these extracts at 663 and 460 nm respectively using the specific extinction coefficients of MacKinney (1941) of 82.04 for chlorophyll a and 200 for total carotenoid content. These results were expressed as fg chlorophyll a or total carotenoid cell^{-1} and as a percentage of the cell dry weight.

2.6.3.2. Phycocyanin determination

The phycocyanin content of the organisms was determined on a known volume of the original suspension (section 2.6.3.1.). The organisms, resuspended in distilled water, were broken by two passages through a French pressure cell (Aminco, Silver Springs, Maryland, U.S.A.) at 8.3 x 10^7 Pa (12,000 lbf in^{-2}) at 4°C, followed by centrifugation (15,000 x g for 20 min) to remove cell debris and any unbroken organisms. Samples were taken before and after disruption for cell number determination and the estimation of cell breakage. The absorption spectrum was determined on the resulting blue-green supernatant using a distilled water blank and the secondary position in the spectrophotometer. (This position was closer to the photomultiplier of the instrument so reducing light scattering in

a similar way to the opal glass method of Shibata, Benson and Calvin (1954)). The phycocyanin concentration was determined from this extract using the equation of Myers and Kratz (1955) in which correction was made for chlorophyll absorption:

$$OD \text{ phycocyanin} = 1.016\ OD_{618} - 0.203\ OD_{677}$$

The phycocyanin content of the cells was determined using the specific extinction coefficient of Svedburg and Katsurai (1929) of 7.9 at 618 nm.

The phycocyanin content of the organisms was also determined on the blue cells remaining after acetone extraction which were resuspended in distilled water and the spectrum determined as above.

These results were expressed as pg phycocyanin $cell^{-1}$ and as a percentage of the cell dry weight.

2.6.4. Carbohydrate determination

Steady state cultures were harvested, washed and resuspended in a known volume of distilled water to give an absorbance of approximately 1.5 at 600 nm. Carbohydrate determinations were carried out on the suspension by two different methods - the anthrone method (Morris, 1948) as modified by Herbert, Phipps and Strange (1971) and the phenol method of Dubois, Gilles, Hamilton, Rebers and Smith (1956).

2.6.4.1. Anthrone method

A series of thin-walled boiling tubes were set up containing 0.25 to 1.0 ml of the washed cell suspension and made up to 1.0 ml

if necessary with distilled water. A reagent blank was also prepared containing 1.0 ml distilled water.

The tubes were cooled by standing in ice-water and the fresh anthrone reagent also cooled. The anthrone reagent contained 200 mg anthrone added to 5.0 ml absolute ethanol and made up to 100 ml with 75% (v/v) H_2SO_4. This was shaken until all the anthrone had dissolved. (The incorporation of ethanol in the reagent was due to Fales (1951) who found that it stabilised the colour, which otherwise tended to fade on standing). When cooled 5.0 ml of the anthrone reagent was added to each tube in turn from a fast flowing pipette, the tube being kept submerged in the ice-water and swirled during this addition. When all the tubes had been treated and all had recooled to 0°C they were transferred to a vigorously boiling water bath. After exactly 10 min, the tubes were returned to the ice bath and when cool the absorbance was determined in a Unicam SP 1700 spectrophotometer at 625 nm using 1 cm pathlength cuvettes against the reagent blank.

A standard curve was prepared using glucose at concentrations of 25 - 100 µg and from this the carbohydrate content of the organisms was estimated and expressed as pg carbohydrate $cell^{-1}$ and as a percentage of the cell dry weight.

2.6.4.2. Phenol method

A series of thick-walled test tubes of 16 - 20 mm diameter were set up containing 0.25 - 1.0 ml of the washed cell suspension

and made up to 1.0 ml if necessary with distilled water. A reagent blank was also prepared containing 1.0 ml distilled water.

1.0 ml 5% (w/v) phenol in distilled water was added to each tube and mixed. 5.0 ml concentrated H_2SO_4 (sp.gr. 1.84) was added from a fast flowing pipette, the stream of acid being directed onto the surface of the liquid and the tube shaken simultaneously to effect fast and complete mixing. (This method employed the 'heat of mixing' technique which did not perform very well with the anthrone reagent. Presumably the phenol reagent was less sensitive than the anthrone reagent to small differences in temperature and heating time). The samples were allowed to stand for 10 min, shaken and placed in a water bath at 27°C for 15 min. The absorbance was determined in a Unicam SP 1700 spectrophotometer at 488 nm using 1 cm pathlength cuvettes against the reagent blank.

A standard curve was prepared using glucose at concentrations of 25-100 µg and from this the carbohydrate content of the organisms was estimated and expressed in terms of pg carbohydrate $cell^{-1}$ and as a percentage of the cell dry weight.

2.6.5. Lipid determination

Steady state cultures were harvested, washed and resuspended in a known volume of distilled water to give an absorbance of approximately 2.5 at 600 nm. Determinations of total lipid content were carried out on this suspension by a modification

of the method of Folch, Lees and Sloane Stanley (1957).

10.0 ml of the cell suspension was centrifuged at 38,000 x g for 15 min and the supernatant discarded. Lipids were extracted from the resulting pellet by the addition of 4.0 ml 2:1 (v/v) chloroform:methanol mixture and incubated at room temperature for an hour. 1.0 ml methanol was added and the sample centrifuged at 38,000 x g for 15 min. (Methanol was added to lower the specific gravity of the extract so that the suspended material would be precipitated. Centrifugation of the original suspension was unsatisfactory as the specific gravity of the solvent mixture was too close to the density of the suspended material). The supernatant fluid was collected after centrifugation and 2.0 ml chloroform added to restore the 2:1 (v/v) ratio of chloroform:methanol. The crude extract was mixed thoroughly with 0.2 of its volume of 0.04% (w/v) aqueous $CaCl_2$. (It was found by Folch et al. (1957) that a virtual absence of lipids could be obtained from the resulting upper phase by the addition of an appropriate salt solution. Apparently, these salts ($CaCl_2$, $MgCl_2$, NaCl or KCl could be used) altered the distribution of lipids and practically eliminated them from the upper phase. In the absence of salts, substantial amounts of acidic lipids were present in the upper phase and were lost during the washing procedure). The sample was centrifuged at 38,000 x g for 20 min to form two phases without interfacial debris. (Centrifugation was found to be a more effective means of separation of the two phases than leaving the sample to stand for several hours to separate out.) The

resulting upper phase contained all of the non-lipid substances and only negligible amounts of other lipids whereas the lower phase contained essentially all of the cell lipids. Consequently, as much of the upper phase as possible was removed by very careful use of a Pasteur pipette and removal of the solutes was completed by rinsing the interface three times with small amounts (approximately 1.5 ml) of 'pure solvents upper phase' (a mixture of 3:48:47 by volume of chloroform:methanol:water respectively). The inside wall of the tube was rinsed and the solution allowed to flow gently from the pipette so that the washing fluid collected on top of the lower phase without mixing of the two phases. The tube was rotated gently so as to ensure mixing of the rinsing fluid with the remaining original upper phase and the mixture was removed with a Pasteur pipette. After washing, the lower phase and remaining rinsing fluid were made into a single phase by the addition of approximately 0.5 ml methanol. This was transferred to a tared 2 dram vial and the tube washed twice with 0.5 - 1.0 ml chloroform: methanol (2:1 (v/v)) mixture and the washings added to the vial. The solution was evaporated under a stream of nitrogen to prevent oxidation of the lipid products and the vial transferred to a vacuum dessicator where the residue was dried over phosphorus pentoxide until constant weight was achieved. The amount of cell dry weight as lipid extractable material was calculated from the dry weight of the residue and the results expressed in terms of pg lipid $cell^{-1}$ and as a percentage of the cell dry weight.

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2.7. ULTRASTRUCTURE STUDIES

2.7.1. Fixation

A modification of the method of Kellenberger, Ryter and Sechaud (1958) was used for the fixation of samples for ultrastructure studies of A. nidulans.

Approximately 8 x 10^9 cells were harvested from steady state cultures, washed twice and resuspended in 10.0 ml Kellenberger buffer. (The Kellenberger buffer contained 5.0 ml veronal acetate buffer (2.94 g sodium veronal, 1.94 g sodium acetate (trihydrate) and 3.40 g sodium chloride in 100 ml distilled water), 13.0 ml distilled water, 7.0 ml 0.1N HCl and 0.25 ml 1.0M $CaCl_2$. The pH was adjusted to 6.0 with the HCl, the buffer being prepared the day it was required). 1.0 ml (that is, 0.1%) Kellenberger fixative (01g OsO_4 in 10.0 ml Kellenberger buffer prepared in concentrated HNO_3 washed glassware) was added for prefixation and the sample immediately centrifuged at 2990 x g for 5 min. The supernatant was discarded and the pellet resuspended in 2.0 ml Kellenberger fixative plus 0.2 ml (0.1 ml for each 1.0 ml of fixative) fresh tryptone medium (1.0 g Difco Bacto-tryptone and 0.5 g NaCl in 100 ml distilled water) and placed in an acid washed brown glass stoppered bottle which was covered with foil. This was left for 18 hours in the dark at room temperature. The resulting black suspension was carefully transferred to a centrifuge tube, diluted with 8.0 ml Kellenberger buffer and centrifuged at 2990 x g for 10 min. The

supernatant was discarded and the pellet resuspended in the smallest possible amount of 2% (w/v) agar (TAAB, high purity agar). This was mixed well using a Pasteur pipette and placed as a drop on a microscope slide ensuring that no air bubbles were present. (These manipulations with the agar had to be carried out at 45°C to prevent it from setting too quickly.) After cooling and gelation on the slide the agar was sliced into cubes of 1 mm square. The blocks of embedded cells were placed in fresh uranyl acetate washing fluid (0.5 g uranyl acetate in 100 ml Kellenberger buffer) for 2 hours at room temperature and washed twice, for approximately 15 min each, with Kellenberger buffer.

2.7.2. Dehydration

The blocks were dehydrated in a graded series of ethanol concentrations using the following procedure: 30% (v/v) aqueous ethanol for 15 min, 50% (v/v) aqueous ethanol for 15 min, 70% (v/v) aqueous ethanol for 30 min, 90% (v/v) aqueous ethanol for 30 min and absolute alcohol for 60 min (including at least 2 changes).

2.7.3. Embedding

Once dehydration was complete, the blocks were placed in equal amounts of absolute alcohol and Spurr resin (Spurr, 1969) for two changes of 30 min and 5 hours and then in Spurr resin only overnight. (Spurr resin and alcohol were miscible so intermediate changes with, for example, propylene oxide, were not needed in contrast to the use of Araldite, for example, as

the embedding resin). The Spurr resin contained nonenyl succinic anhydride (NSA) as the hardener, vinyl cyclohexene dioxide (ERL 4206) as the resin, diglycidyl ether of polypropylene glycol (DER 736) as the plasticiser and dimethylaminoethanol (S-1) as the accelerator. The components were mixed together in the following volumes: 10.0 g ERL 4206, 6.0 g DER 736, 26.0 g NSA and 0.4 g S-1. The blocks sunk in the resin overnight indicating that they had been properly dehydrated and they were placed into fresh Spurr resin for about 30 min before being embedded. Each block was carefully placed at the base of a BEEM capsule 00 (Agar Aids, Stansted, Essex) which was filled with the resin and cured for 8 hours at 70°C. Once the resin block had hardened the plastic capsules were cut away leaving the solid block of resin ready for sectioning.

2.7.4. Sectioning and microscopy

Thin sections of the hardened resin block were cut with a diamond knife using an ultramicrotome (Om U2 microtome, Reichert, Austria), floated on distilled water, expanded with chloroform and, as viewed by reflected light, were picked up on uncoated 400 mesh copper grids (Agar Aids, Stansted, Essex) which had been washed in 0.1M HCl.

The material was examined in an AEI Corinth 275 electron microscope (Kratos, Manchester) at an accelerating voltage of 60 kv and photographs of the resulting cell sections were taken using Ilford Line Film.

2.8. LIPID STAINING

Due to the results obtained from the ultrastructure studies it was thought that the cells growing under carbon dioxide-limited conditions at D=0.02 h^{-1} might contain large lipid inclusions so the lipid stain of Burdon, as stated by Cruickshank, Duguid, Marmion and Swain (1975) was carried out on these organisms. A film of the cell suspension was prepared on a microscope slide, dried in air and fixed by flaming. The entire slide was covered with Sudan black stain (0.3 g Sudan black B powder in 100 ml 70% (v/v) aqueous ethanol which was shaken thoroughly at regular intervals and left to stand overnight before using) and left at room temperature for 15 min. The excess stain was drained off and the sample blotted and dried in air. When dry it was rinsed thoroughly with xylol and again blotted dry. This was counterstained lightly by covering the slide with 0.5% (v/v) aqueous safranine for 5 - 10 seconds, rinsed with tap water, blotted and dried.

Lipid inclusion granules stain blue-black or blue-grey, whilst bacterial cytoplasm stains light pink when examined by light microscopy.

2.9. CARBOXYLASE DETERMINATIONS

The activities of three carboxylase enzymes were determined in A. nidulans - ribulose 1,5-bisphosphate carboxylase, RuBPCase, (3-phospho-D-glycerate carboxylyase (dimerizing) E.C.4.1.1.39), phosphoenol pyruvate carboxylase, PEPCase, (orthophosphate: oxaloacetate carboxylyase (phosphorylating) E.C.4.1.1.31) and

pyruvate carboxylase (pyruvate:CO_2 ligase (ADP) E.C. 6.4.1.1.). The enzymes were assayed at 40°C (the growth temperature) at optimum pH, substrate and cofactor concentrations.

Steady state cultures were harvested, washed and resuspended in a known volume of ice-cold 0.02M Tris-HCl buffer, pH 7.5, to give 1.0 - 2.0 mg protein ml^{-1}. Two different methods of assaying these enzymes were used - a modification of the filter method of Glover and Morris (1979) and a modification of the method of Slater (1975). In the former method the cells were disrupted by using a 10% (v/v) aqueous solution of Triton X-100 (a detergent) and in the latter method by two passages through the French pressure cell at 8.3×10^7 Pa at 4°C. In the latter case the disrupted cell suspension was centrifuged at 38,000 x g for 45 min at 4°C to remove cell debris and unbroken organisms and the resulting cell-free extract was used for the enzyme assays. Samples were taken before and after disruption for cell number determination and estimation of the cell breakage.

Whole cell samples and cell-free extract samples were stored at -20°C for subsequent protein determination (section 2.6.2.)

2.9.1. Ribulose 1,5-bisphosphate carboxylase determination

0.4 ml 10% (v/v) aqueous solution Triton X-100 was added to 0.1 or 0.2 ml whole cell suspension and incubated at 40°C for 20 min. Meanwhile, cell-free extract samples of 0.1 or 0.2 ml were also set up. 0.9 ml RuBPCase mix containing 210 µmol Tris-HCl buffer, pH 7.8 (the optimum pH for the activity of this

enzyme having been determined by Karagouni (1979)), 5 µmol reduced glutathione, 10 µmol $MgCl_2$ and 25 µmol $NaH^{14}CO_3$ (specific activity, 1 µCi μmol^{-1}) was added to all samples which were incubated for 10 min at 40°C. This incubation time allowed the samples to equilibrate before the reaction was started by the addition of 5.16 µmol ribulose 1,5-bisphosphate. This was prepared from the dibarium salt by adding a small quantity of Dowex 50 ion exchange resin and equilibrating for 10 min. The water insoluble salt was converted to the free acid and was neutralised to approximately pH 7.0 with 2N sodium hydroxide. A control system was set up with each set of samples to which no substrate was added.

0.1 ml aliquots were taken from each sample at suitable time intervals up to 20 min after the addition of the substrate and the reaction terminated in 0.2 ml 95% (v/v) ethanol: 5% (v/v) acetic acid in scintillation vials. The contents of the vials were evaporated to dryness in a stream of air. This process reduced the aqueous content of the sample necessary for the scintillation system used and also ensured the removal of any unassimilated sodium [^{14}C] - bicarbonate. Therefore, only [^{14}C] - carbon assimilated into cellular material remained in the vial and the radioactivity was determined as described in section 2.9.4.

The activity of ribulose 1,5-bisphosphate carboxylase was expressed as µmol CO_2 fixed h^{-1} (mg protein)$^{-1}$ and as µmol CO_2 fixed h^{-1} (10^8 organisms)$^{-1}$.

2.9.2. Phosphoenol pyruvate carboxylase determination

0.4 ml 10% (v/v) aqueous solution Triton X-100 was added to 0.1 or 0.2 ml cell suspension and incubated at 40°C for 20 min. Meanwhile, cell-free extract samples of 0.1 or 0.2 ml were also set up. 1.0 ml PEPCase mix was added to each sample plus 0.3 µmol acetyl-CoA (lithium salt) and these were incubated at 40°C for 10 min. The mix contained, ml^{-1}: 200 µmol Tris-HCl buffer, pH 8.2 (this was the optimum pH for activity of this enzyme as shown in figure 2.3.), 10 µmol $MgCl_2$, 5 µmol reduced glutathione, 20 µmol sodium glutamate, 50 Units glutamate oxaloacetic transaminase and 25 µmol $NaH^{14}CO_3$ (specific activity, 1 µCi μmol^{-1}). The reaction was started after the equilibration time by the addition of 36.5 µmol phosphoenol pyruvate (trisodium salt), pH 7.0, and sampling and radioactivity determinations were carried out in the same way as for the RuBPCase assay.

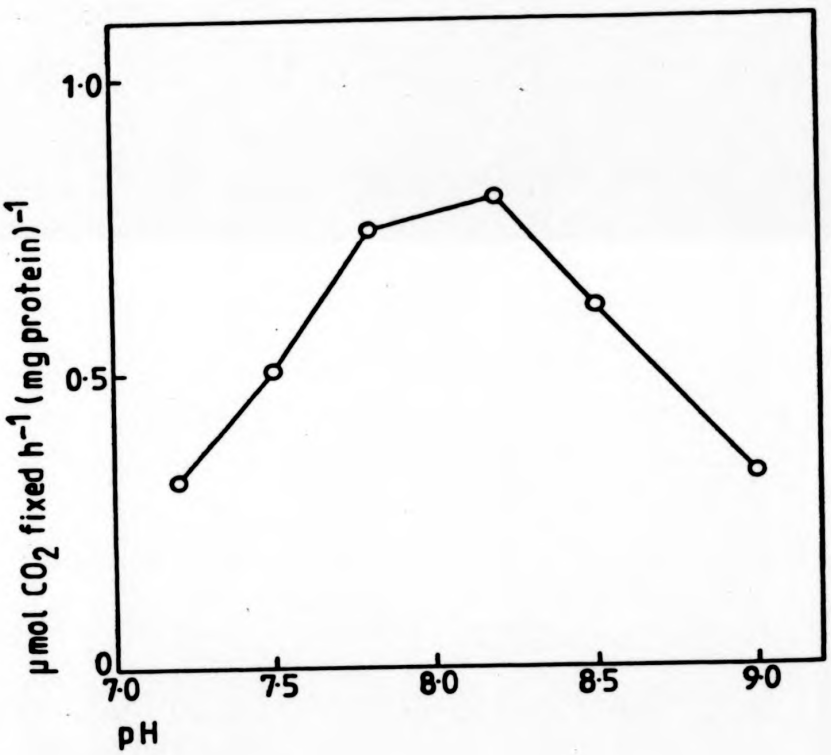
The activity of phosphoenol pyruvate carboxylase was expressed as µmol CO_2 fixed h^{-1} (mg protein)$^{-1}$ and as µmol CO_2 fixed h^{-1} (10^8 organisms)$^{-1}$.

2.9.3. Pyruvate carboxylase

0.4 ml 10% (v/v) aqueous solution Triton X-100 was added to 0.1 or 0.2 ml cell suspension and incubated at 40°C for 20 min. Meanwhile, cell-free extract samples of 0.1 or 0.2 ml were also set up. 1.0 ml pyruvate Case mix was added to each sample plus 0.3 µmol acetyl-CoA and these were incubated at 40°C for 10 min. The mix contained, ml^{-1}: 200 µmol Tris-HCl buffer, pH8.2, 10 µmol

Figure 2.3.

pH curve for PEP carboxylase activity. The activity of the enzyme, after cell disruption by the Triton method, measured at different pH values in Tris-HCl buffer.


1·0
0·5
0
μmol CO_2 fixed h^{-1} (mg protein)$^{-1}$
7·0
7·5
8·0
8·5
9·0
pH

$MgCl_2$, 5 µmol reduced glutathione, 1 µmol adenosine triphosphate (ATP), 50 Units malate dehydrogenase and 25 µmol $NaH^{14}CO_3$ (specific activity, 1 µCi μmol^{-1}). The reaction was started after the equilibration time by the addition of 30 µmol pyruvate (sodium salt) and sampling and radioactivity determinations were carried out in the same way as for the RuBPCase assay.

The activity of pyruvate carboxylase was expressed as µmol CO_2 fixed h^{-1} (mg protein)$^{-1}$ and as µmol CO_2 fixed h^{-1} (10^8 organisms)$^{-1}$.

2.9.4. Counting of [^{14}C] - radioactivity.

The dried vials were filled with 15.0 ml of a scintillation fluid containing 6.0 g 2-(4'tert-butylphenyl)-5-(4"-biphenylyl)-1,3,4-oxadiazole (Butyl-PBD) in 750 ml sulphur-free toluene and 250 ml methanol and the radioactivity determined using a Packard 2425 liquid scintillation counter.

Standard vials with a known number of disintegrations per minute (dpm) were used regularly to check the counting efficiency of the instrument. These were prepared using a standard n-hexadecane-1-[^{14}C] solution with an activity of 1.1 µCi g^{-1} (1 µCi [^{14}C] being equivalent to 2.2×10^6 dpm). The average efficiency of counting for the scintillant used was 81% (table 2.1.). Similar automatic external standard readings were obtained for the sample vials so it was assumed that the efficiency of counting was the same as for the standards.

TABLE 2.1. Determination of the counting efficiency of the scintillation counter using n-hexadecane standards

n-hexadecane 1.1 μCi g^{-1} (g)	Expected disintegrations per minute (dpm)	Observed counts per minute (cpm)	Efficiency (%)
0.00398	9632	7491	77.8
0.00978	23668	19764	83.5
0.01143	27661	23306	84.3
0.09464	229029	181860	79.4
0.09963	241105	193485	80.3

2.10 COMPETITION STUDIES

2.10.1. Growth medium

The two organisms used in these studies, A. nidulans and S. quadricauda, were normally cultured in different growth media, Medium C and GAM respectively (section 2.2.), so a growth medium for these experiments had to be determined which did not favour the growth of one organism over the other. Batch growth experiments were therefore carried out for both organisms in both types of media by the use of Dreschel bottles sparged with a continuous supply of 5% CO_2:95% air (v/v) (as green algae need aerobic conditions for growth). These were kept at a temperature of about 27°C (as this was about the optimum temperature for the growth of S. quadricauda and the optimum temperature for the growth of A. nidulans could not be used as the green alga could not survive at 40°C) and were supplied with a constant light source (a 100 W tungsten filament light bulb) placed 15 cm from the Dreschel bottle.

S. quadricauda grew slightly better in Medium C than in GAM (the μ_{max} values being 0.037 and 0.032 h^{-1} respectively) and A. nidulans grew very much better in Medium C than in GAM (the μ_{max} values being 0.058 h^{-1} and 0.032 h^{-1} respectively). Consequently, Medium C plus thiamine (a vital vitamin requirement for many green algae) was used as the growth medium in the chemostat for these experiments.

2.10.2. Continuous-flow culture system for mixed culture studies

The light-limited continuous-flow culture system used in

these studies was essentially the same as that previously described (section 2.4.2. and figure 2.1.), except that the culture within the growth vessel had a working volume of 1.0 l and was illuminated on two sides by six 8 W Warm White fluorescent tubes placed 10 cm from the culture vessel plus two 100 W tungsten filament light bulbs placed 15 cm behind the culture vessel.

The culture was continuously sparged with 5% CO_2:95% air (v/v) and the temperature within the culture vessel controlled to 27°C with an accuracy of approximately ±0.5°C by a combination of the cooling finger supplied with water at a temperature of 10°C from a chiller Churchill (Churchill Chiller Thermocirculator, Middlesex) and the heater element of the combined magnetic stirrer/heater which was thermostatically controlled as previously described (section 2.4.2.). The growth medium used was as described in section 2.10.1.

This system was also used for the carbon dioxide-limited growth studies due to the addition of pH monitoring and controlling facilities as described in section 2.4.3. and shown in figure 2.2. As previously described the pH was maintained at a value of 7.5 ± 0.2 (section 2.4.3.). Under these conditions the culture was continuously sparged with air only and the growth medium used was as described in section 2.10.1. supplemented with $NaHCO_3$ to give a final concentration of 0.42 g l^{-1} (5 mM) as described in section 2.4.1.2.

2.10.3. Inoculation

Inocula for these studies were grown in Dreschel bottles under the conditions of light and temperature previously described (section 2.10.1.) and exponentially growing cultures were added to the growth vessel. It was important to have cultures of both organisms in a similar state of growth for inoculation so that one organism was not initially favoured over the other.

The growth vessel was inoculated aseptically through one of the inlet ports. Under light-limited conditions the organisms were initially grown as a batch culture and the fresh medium flow was started as soon as the culture was growing exponentially (measured by absorbance at 650 nm). Under carbon dioxide-limited conditions, however, the medium flow had to be started almost immediately due to the difficulty in establishing cultures under these conditions (section 2.4.4.2.).

2.10.4. Basic growth characteristics

Basic growth characteristics, in terms of absorbance, dry weight and cell number (sections 2.5.2., 2.5.3. and 2.5.4. respectively) were determined for each organism separately under light-limited conditions on steady state cultures over an appropriate growth range which had been previously determined from batch growth curves. This was to determine whether A. nidulans showed similar basic growth characteristics under these conditions as shown in the other light-limited chemostat and to determine the basic growth characteristics of S. quadricauda which had not previously been grown under continuous-flow conditions.

2.10.5. Competition experiments

In most experiments the two organisms were inoculated into the chemostat on a 50:50 dry weight basis, although a few experiments were carried out using unequal biomass concentrations to see whether or not this affected the results. Experiments were carried out under light-and carbon dioxide-limited conditions at dilution rates of 0.025, 0.035 and 0.045 h^{-1} to determine the best competitor under each condition.

The chemostat was inoculated as previously described (section 2.10.3) and growth of the organisms was followed by total biomass determinations, in terms of absorbance at 650 nm and dry weight (mg ml^{-1}), and differential counts of individual cells using the Neubauer counting chamber. (This method of counting the organisms when they were mixed together was found to be the most accurate, although a number of other methods were tried, for example, heat fixing the organisms on slides and staining with Methylene blue, Coulter counting and filtering.) The experiments were stopped once one of the species had been virtually eliminated from the culture or when a steady state of both organisms had been achieved.

2.11. MATERIALS

The medium components were all of analytical grade and were obtained from British Drug Houses (Poole, Dorset) and Fisons Limited (Loughborough, Leicestershire). The 'lab m' and nutrient agar were obtained from London Analytical and Bacteriological Media Limited (London) and the high purity agar used for the

electron microscopy studies from TAAB (Reading). All acids and ethanol, acetaldehyde, phenol, chloroform, methanol and toluene were obtained from Fisons Limited as well as KCl, NaCl, $NaCO_3$, NaOH, $MgCl_2$, diphenylamine, orcinol, D-glucose and sodium acetate, all of which were of analytical grade except for orcinol and acetaldehyde which were standard laboratory reagents. Sodium potassium tartrate, $FeCl_3$ and $CaCl_2$ of analytical grade were obtained from British Drug Houses as well as the standard laboratory reagents glutaraldehyde and Folin and Ciocalteu's phenol reagent plus the scintillation grade reagent Butyl-PBD and the stains Sudan black B, xylol and safranine. Sodium veronal, osmium tetroxide, uranyl acetate and the Spurr resin kit were obtained from Agar Aids (Stansted, Essex) and the Difco Bacto-tryptone from Difco (Detroit, Michigan). The thiamine, 2-deoxy-D-ribose, D(-)ribose, bovine serum albumin and anthrone were obtained from Sigma (London) Chemical Co. as well as ribulose 1,5-bisphosphate, phosphoenol pyruvate, pyruvate, the Tris bases, Triton X-100, reduced glutathione, Dowex, acetyl-CoA, sodium glutamate, glutamate oxaloacetic transaminase, ATP and malate dehydrogenase. The radioactive substrate $NaH^{14}CO_3$ and the standard n-hexadecane-1-^{14}C solution were obtained from the Radiochemical Centre (Amersham, Bucks.).

PART 3. BASIC GROWTH CHARACTERISTICS FOR ANACYSTIS NIDULANS UNDER CHEMOSTAT CONTINUOUS-FLOW CULTURE CONDITIONS

3.1. GENERAL MORPHOLOGY

Chemostat cultures of A. nidulans were examined regularly under the light microscope and it was seen that the organisms were rod shaped and usually occurred in pairs, although infrequently chains of cells up to 4 in length were seen. However, under certain conditions, especially at low dilution rates in light-limited chemostat cultures, chains of cells up to 8 or 10 in length occurred.

3.2. ELEMENTAL ANALYSIS

The elemental analysis for A. nidulans grown under light-limited conditions showed that the organisms contained on a dry weight basis (w/w) 47.6% carbon, 11.1% nitrogen and 6.9% hydrogen.

3.3. GROWTH UNDER LIGHT-LIMITED CONDITIONS

A. nidulans was grown in a light-limited chemostat as described in section 2.4.2. The culture biomass, expressed in terms of absorbance, dry weight (mg ml^{-1}) and cell number (organisms ml^{-1}), and the cell size (pg dry weight $(cell)^{-1}$) were determined in a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The biomass concentration, measured in terms of absorbance and cell number (figure 3.1.) and dry weight (figure 3.2.), decreased rapidly with increasing dilution rate. When the steady state biomass concentration, in terms of dry weight and cell number,

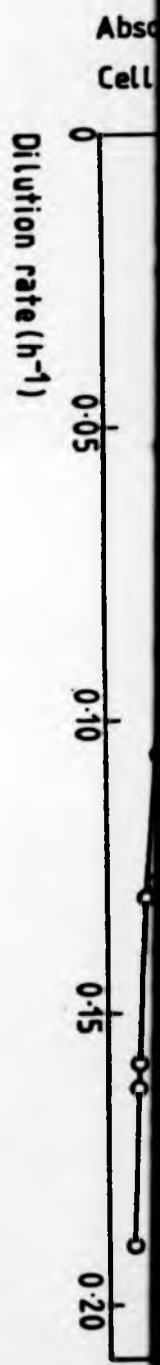


Figure 3.1.

The influence of dilution rate on the steady state biomass concentration, in terms of absorbance (●) and cell number ml^{-1} (○), of A. nidulans grown in light-limited chemostat culture.

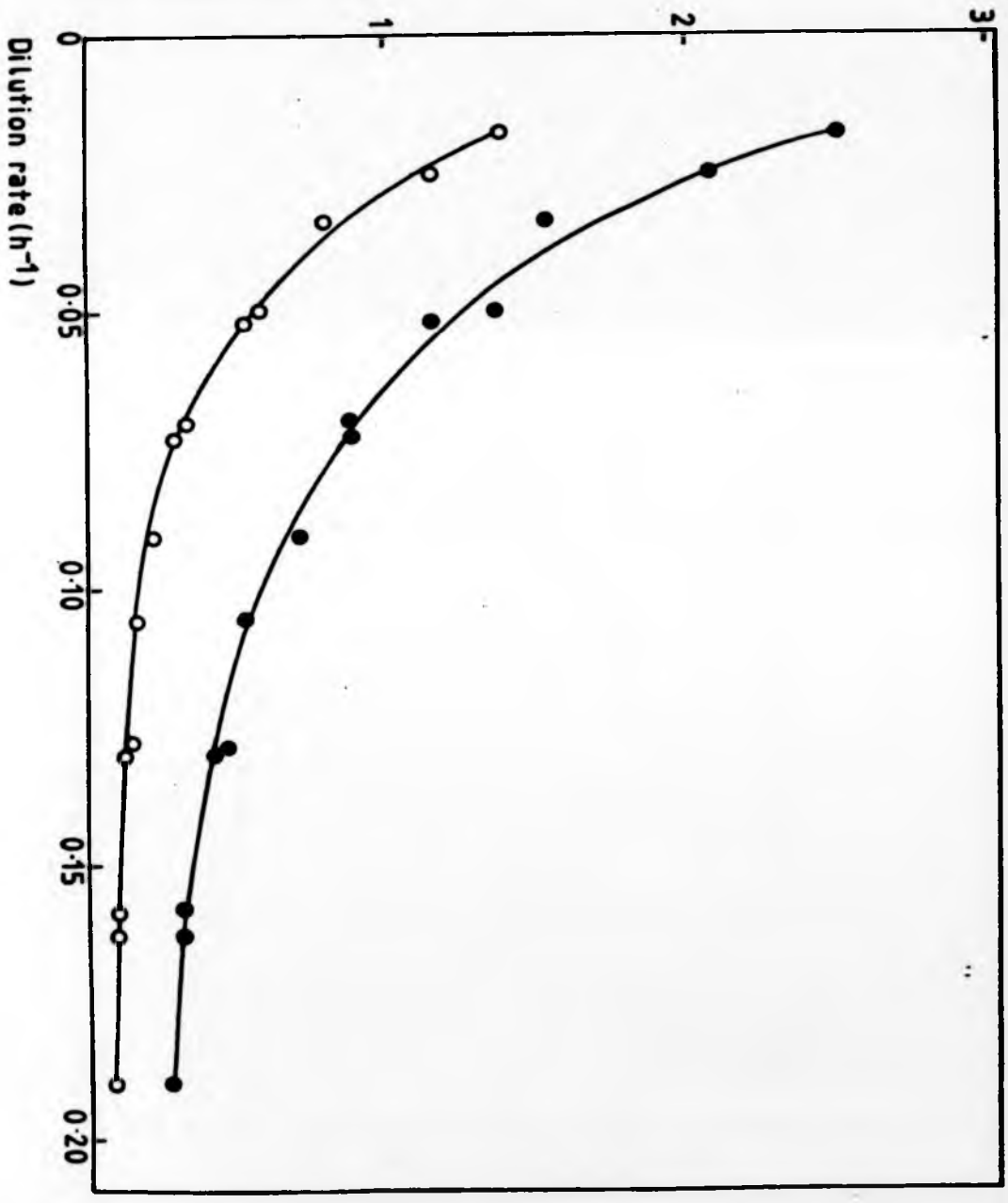
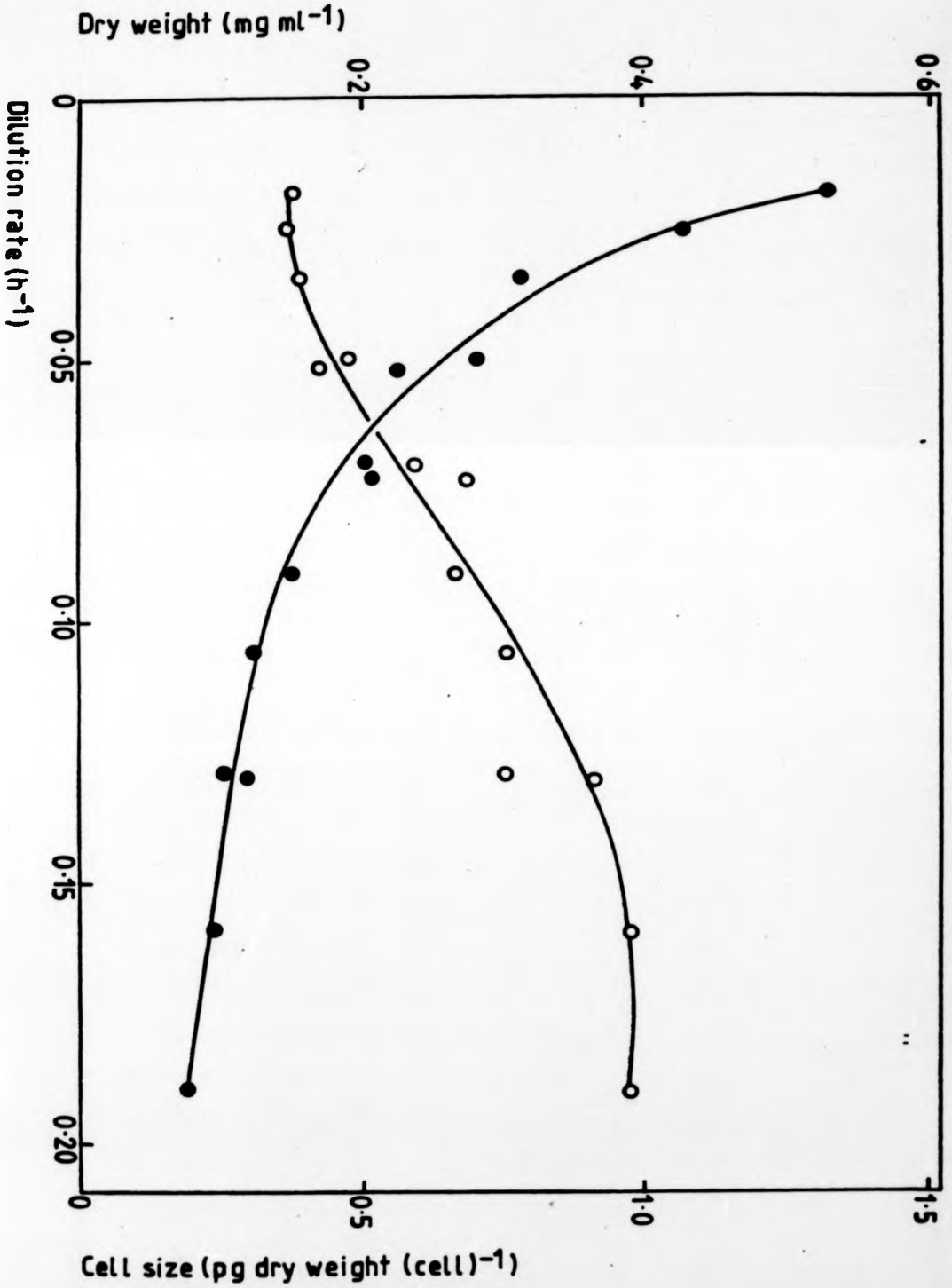

Absorbance (650 nm)
Cell number (ml^{-1} x10^{-9})
1
2
3
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20

Figure 3.2.

The influence of dilution rate on the dry weight, expressed as mg ml^{-1} (●), and cell size, expressed as pg dry weight $(cell)^{-1}$ (○), of A. nidulans grown in light-limited chemostat culture.


Dry weight (mg ml^{-1})
0·2
0·4
0·6
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0
0·5
1·0
1·5
Cell size (pg dry weight (cell)$^{-1}$)

was plotted against $^{1}/_{D}$ (figure 3.3.) a straight line relationship was obtained as predicted in section 1.4.5. However, this relationship was only linear between 0.04 and 0.19 h^{-1} for the dry weight and between 0.025 and 0.19 h^{-1} for the cell number determinations. This indicated that a second nutrient began to limit biomass production at low dilution rates when the biomass concentration was very high. In this case, nitrate was probably the limiting substrate. This was assumed, but not conclusively proven, because of the low levels of the nitrogen-containing pigments, phycocyanin and chlorophyll a, at these low dilution rates (section 4.3.) which would be as expected under nitrogen-limiting conditions (section 1.2.3.).

The dry weight per unit organism increased approximately three-fold as the dilution rate was increased from 0.02 to 0.19 h^{-1} (figure 3.2.) indicating that there was an increase in cell size with increasing growth rate.

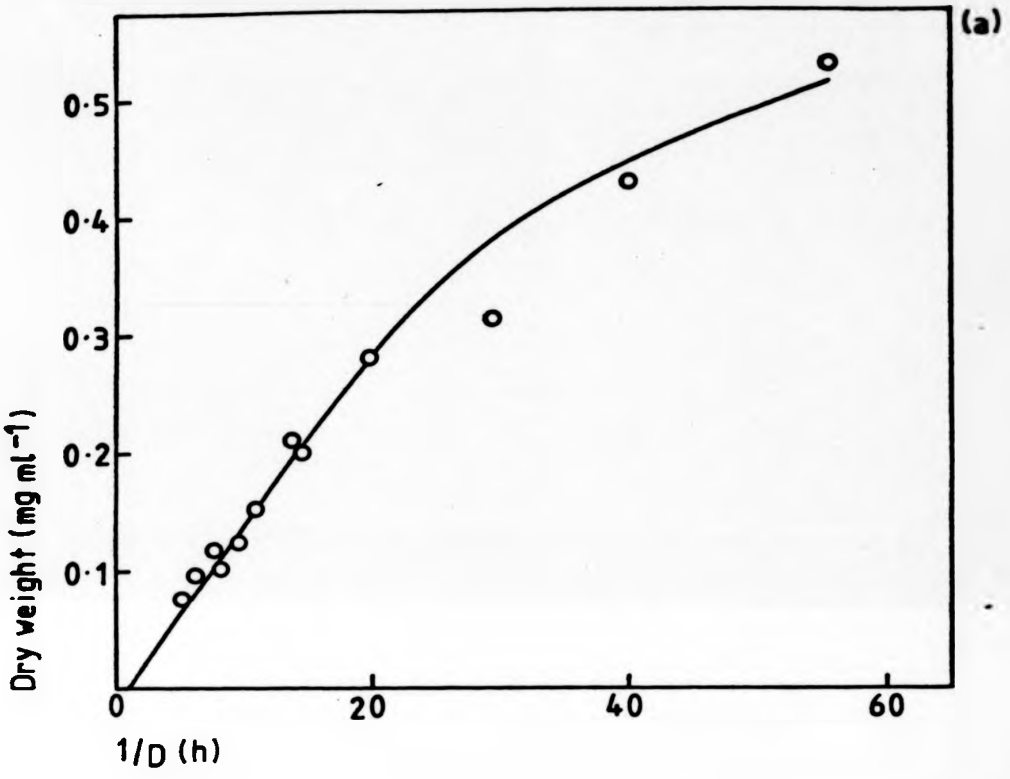
3.4. GROWTH UNDER CARBON DIOXIDE-LIMITED CONDITIONS

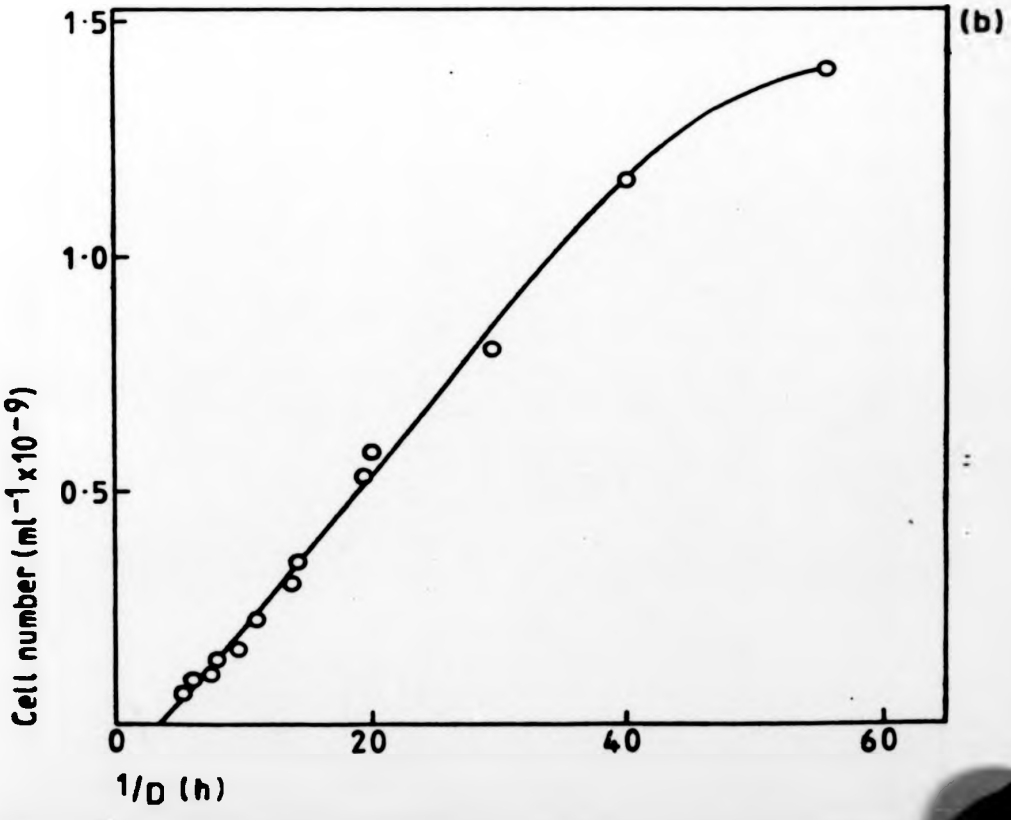
A. nidulans was grown in a carbon dioxide-limited chemostat as described in section 2.4.3. The culture biomass, expressed in terms of absorbance, dry weight (mg ml^{-1}) and cell number (organisms ml^{-1}), and the cell size (pg dry weight $(cell)^{-1}$) were determined in a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The biomass concentration, measured in terms of absorbance and cell number (figure 3.4.) and dry weight (figure 3.5.), showed the normal substrate-limited chemostat pattern of growth (Herbert,

Figure 3.3.

The relationship between the biomass concentration, in terms of (a) dry weight, expressed as mg ml^{-1} and (b) cell number ml^{-1}, and the reciprocal of the dilution rate for A. nidulans grown in light-limited chemostat culture.


(a)
0·5
0·4
0·3
0·2
0·1
Dry weight (mg ml⁻¹)
0
20
40
60
1/D (h)


(b)
1·5
1·0
0·5
Cell number (ml⁻¹ x10⁻⁹)
0
20
40
60
1/D (h)

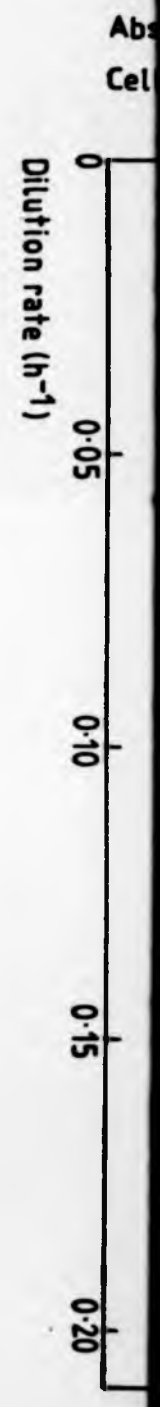
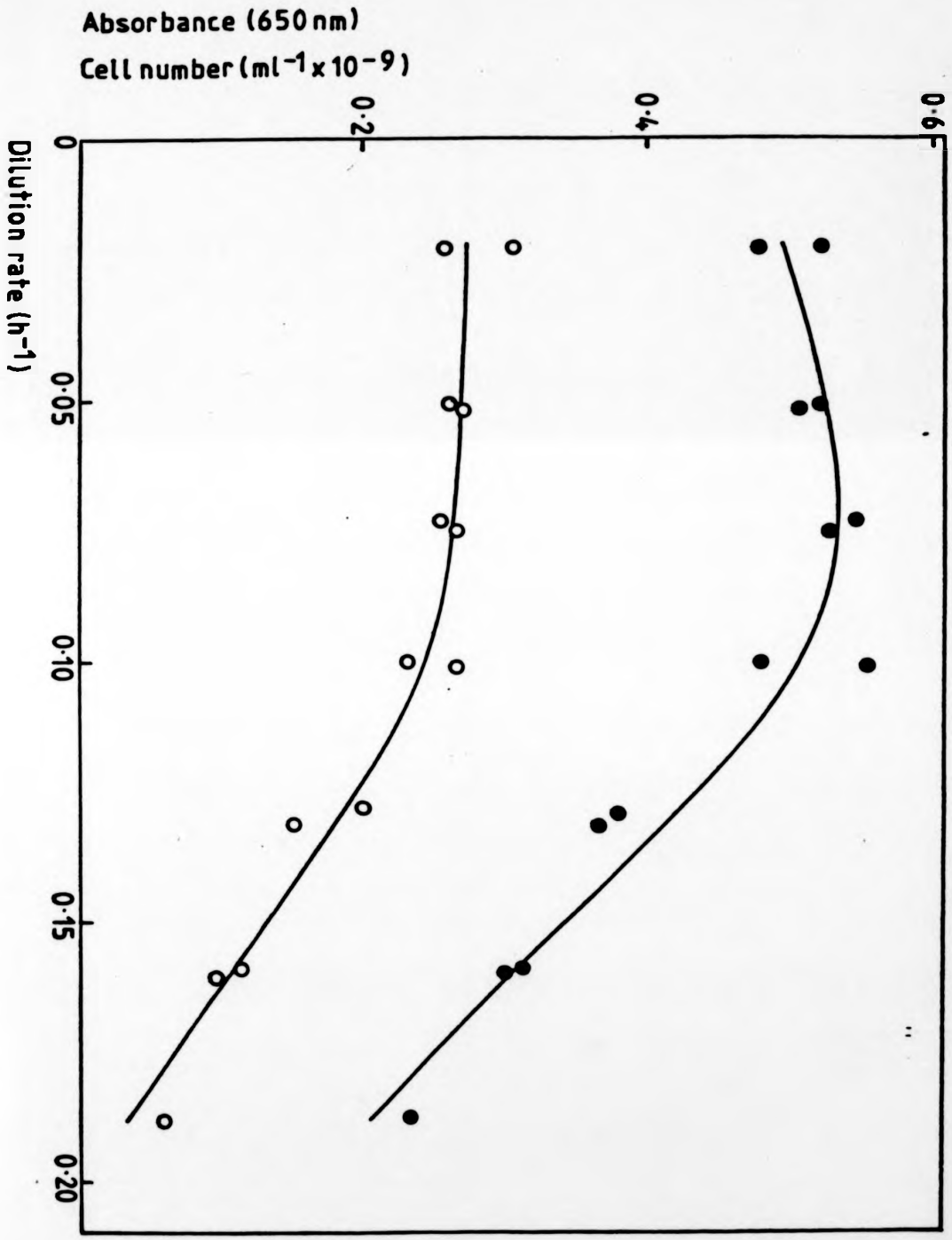


Figure 3.4.

The influence of dilution rate on the steady state biomass concentration, in terms of absorbance (●) and cell number ml^{-1} (○), of A. nidulans grown in carbon dioxide-limited chemostat culture.


Absorbance (650 nm)
Cell number (ml⁻¹ x 10⁻⁹)
0·2
0·4
0·6
Dilution rate (h⁻¹)
0
0·05
0·10
0·15
0·20

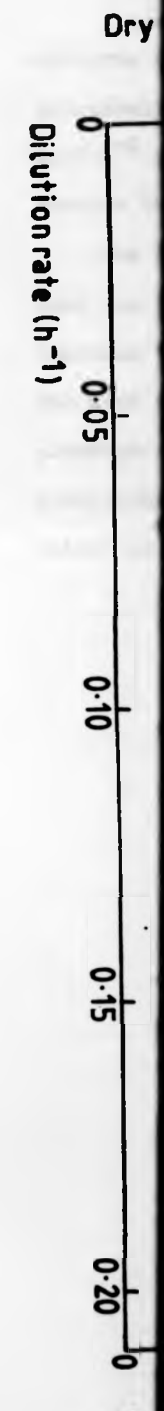
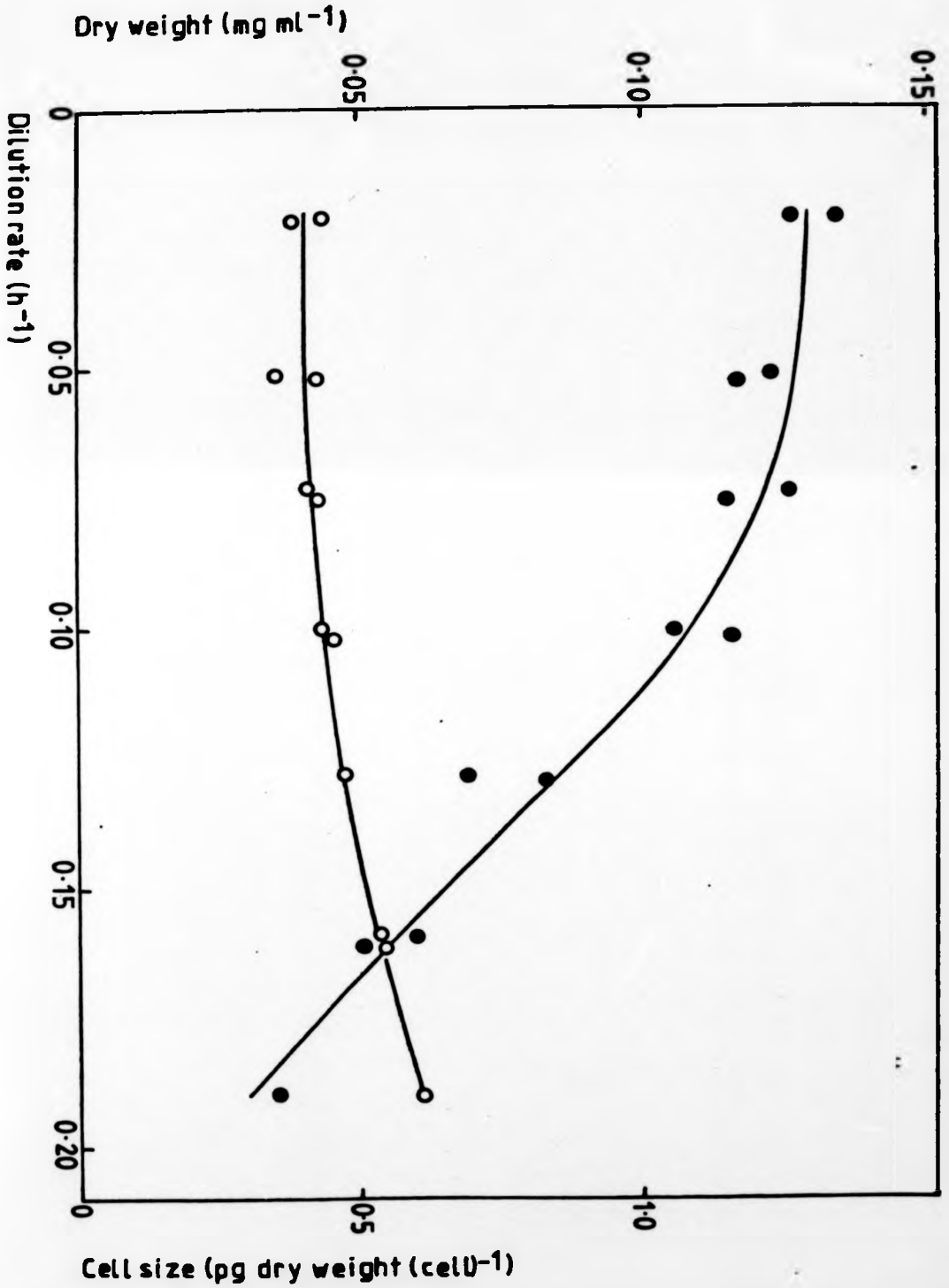


Figure 3.5.

The influence of dilution rate on the dry weight, expressed as mg ml^{-1} (●), and cell size, expressed as pg dry weight $(cell)^{-1}$ (○), of A. nidulans grown in carbon dioxide-limited chemostat culture.


Dry weight (mg ml^{-1})
0·05
0·10
0·15
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0·5
1·0
Cell size (pg dry weight (cell)$^{-1}$)

Elsworth and Telling, 1956) with the culture density remaining relatively constant up to a dilution rate of approximately 0.10 h^{-1} and then decreasing as the dilution rate was increased towards the critical dilution rate.

The dry weight per unit organism increased as the dilution rate was increased (figure 3.5.) indicating that there was an increase in cell size with increasing growth rate. This increase was most marked above a dilution rate of approximately 0.10 h^{-1} although was not as great as that seen under light-limited conditions. Light-limited organisms being almost 50% greater in weight at the highest growth rates.

3.5. DISCUSSION

A. nidulans is normally thought of as a unicellular organism although under certain environmental conditions, as mentioned in the results (section 3.1.), chains of cells can occur. This phenomenon of being able to produce 'filaments' has led to much controversy in the past regarding the classification of this organism. The rod-shaped organism was originally purified by Kratz and Allen and, according to Kratz and Myers (1955), was tentatively identified by Drouet as a member of the Chroococcaceae under the name A. nidulans. Subsequently, however, according to Silva (1962), Drouet reidentified the strain as a member of the Oscillatoriaceae, Phormidium mucicola and more recently in his 'Revision of the Oscillatoriaceae', according to Fogg, Stewart, Fay and Walsby (1973) Drouet placed it as a form of the filamentous Schizothrix calcicola. Pringsheim (1968) also interpreted the chains of cells produced by this organism as trichomes, although of an unusual kind, and proposed the new generic name of Lauterbornia.

On the other hand, a number of workers have concluded that this organism is truly unicellular (Allen and Stanier, 1968; Padmaja and Desikachary, 1968; Stanier, Kunisawa, Mandel and Cohen-Bazire, 1971; Rippka, Deruelles, Waterbury, Herdman and Stanier, 1979) and that chain formation is insufficient ground for excluding this organism from the Chroococcales. Recently, the organism has, in fact, been reclassified as Synechococcus 6301 which is in agreement with the views of Padmaja and Desikachary (1968)

and Stanier et al. (1971) who thought that this organism should be classified in the genus Synechococcus.

So, it seems that the ability of this organism to form chains of cells is a morphological adaptation to certain environmental conditions. This is not a unique situation as other cyanobacteria have also shown changes in morphology due to changing environmental conditions. For example, the availability of reduced carbon substrate, light, nitrogen and temperature all caused alterations in the cell type of Chlorogloea fritschii, two major cell types (irregular clumps of cells (aseriate) and filaments) being distinguished (Evans, Foulds and Carr, 1976), cells of Synechococcus cedrorum grown at 40°C were longer and thinner than those grown at 30°C (Sherman, 1978) and light intensity, temperature and total dissolved solids affected the cell size of the hot spring alga, Synechococcus lividus (Kullberg, 1979).

The elemental analysis obtained for A. nidulans (section 3.2.) was in close agreement with a similar analysis carried out on nitrate-grown cells of the marine cyanobacterium Agmenellum quadriplaticum (van Baalen and Marler, 1963). This analysis showed that the organisms contained on a dry weight basis (w/w) 46.64% carbon, 9.7% nitrogen and 6.58% hydrogen. Slightly different results were obtained when the organism was grown on uric acid as this strain seemed to have a limited ability to degrade this substrate adequately and so was, in effect, nitrogen deficient. In consequence, although the carbon and hydrogen

values were still very similar, the nitrogen value decreased to 2.69%. A similar value of 50% (w/w) for the carbon content of A. nidulans was also obtained by Batterton and van Baalen (1968) and Frischknecht and Schneider (1979).

The results obtained for the growth of A. nidulans in light-limited chemostat culture were in agreement with previous work on the light-limited growth of this organism (Karagouni, 1979; Karagouni and Slater, 1979), of Rhodopseudomonas capsulata (Aiking and Sojka, 1979) and of Oscillatoria agardhii (van Liere and Mur, 1979). This agreement and the fact that a straight line relationship was obtained (except at very low dilution rates) when $\bar{x}$ was plotted against $^{1}/_{D}$, as predicted in section 1.4.5., indicated that the culture was certainly light-limited over the majority of the growth range. At low dilution rates, however, it seemed that a second nutrient was limiting, probably nitrate, and this limitation seemed to affect the dry weight of the organisms to a greater extent than the cell numbers. This would presumably be as expected as the dry weight would be affected virtually immediately by substrate-limitation, especially if that substrate was used in growth or in the manufacture of cell material. If nitrate was, in fact, the limiting substrate then this would certainly be so. The cell numbers would not be expected to be affected so readily unless perhaps the limiting substrate was directly involved in cell division. In this case it seemed that the cells could still divide at the expected rate between D=0.025 and 0.04 h^{-1} even with the dry weight lower than expected. However, eventually

the limiting substrate presumably became limiting for cell division as well as growth as the cell number values were lower than predicted at dilution rates below 0.025 h^{-1}.

The results obtained for the growth of A. nidulans in carbon dioxide-limited chemostat culture were also in agreement with previous work on this organism (Karagouni, 1979; Karagouni and Slater, 1979). A characteristic substrate-limited pattern of growth (Herbert et al., 1956) was shown, indicating nutrient limitation. The amount of bicarbonate added to the medium supply (5 mM) was chosen (Karagouni, 1979) so that this was definitely the limiting factor with all other required nutrients, supplied as Medium C, in excess (section 2.4.1.2.). Consequently, it was assumed that this chemostat was limited by the supply of carbon dioxide which was added in the form of bicarbonate.

Under both limitations the mean organism size, measured in terms of dry weight per cell, increased with increasing growth rate as expected (Herbert, 1961; Mann and Carr, 1974; Slater, 1975). At the lowest dilution rates the cultures contained similar sized organisms but in faster growing cultures the light-limited organisms were almost 50% greater in weight. This was probably due to the fact that carbon dioxide was in excess under light-limited conditions so extra carbon could have been incorporated into the cell as additional material, such as carbohydrate. In fact, the carbohydrate content of light-limited cells was found to be much higher than that of carbon dioxide-limited cells (section 4.4.) which supports this view.

PART 4. MACROMOLECULAR COMPOSITION OF ANACYSTIS NIDULANS GROWN UNDER CHEMOSTAT CONTINUOUS-FLOW CULTURE CONDITIONS

4.1. NUCLEIC ACID COMPOSITION

4.1.1. Light-limited conditions

The DNA and RNA contents of A. nidulans, expressed as fg $(cell)^{-1}$ and as a percentage of the dry weight, were determined on a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The DNA content per cell increased with increasing growth rate reaching a maximum of 4.5 fg DNA $(cell)^{-1}$ at a dilution rate of approximately 0.1 h^{-1} and then decreased with further increases in dilution rate (figure 4.1.). The DNA content on a percentage dry weight basis was constant up to a dilution rate of approximately 0.09 h^{-1} but declined by approximately two fold with increasing growth rate up to 0.19 h^{-1} (figure 4.1.).

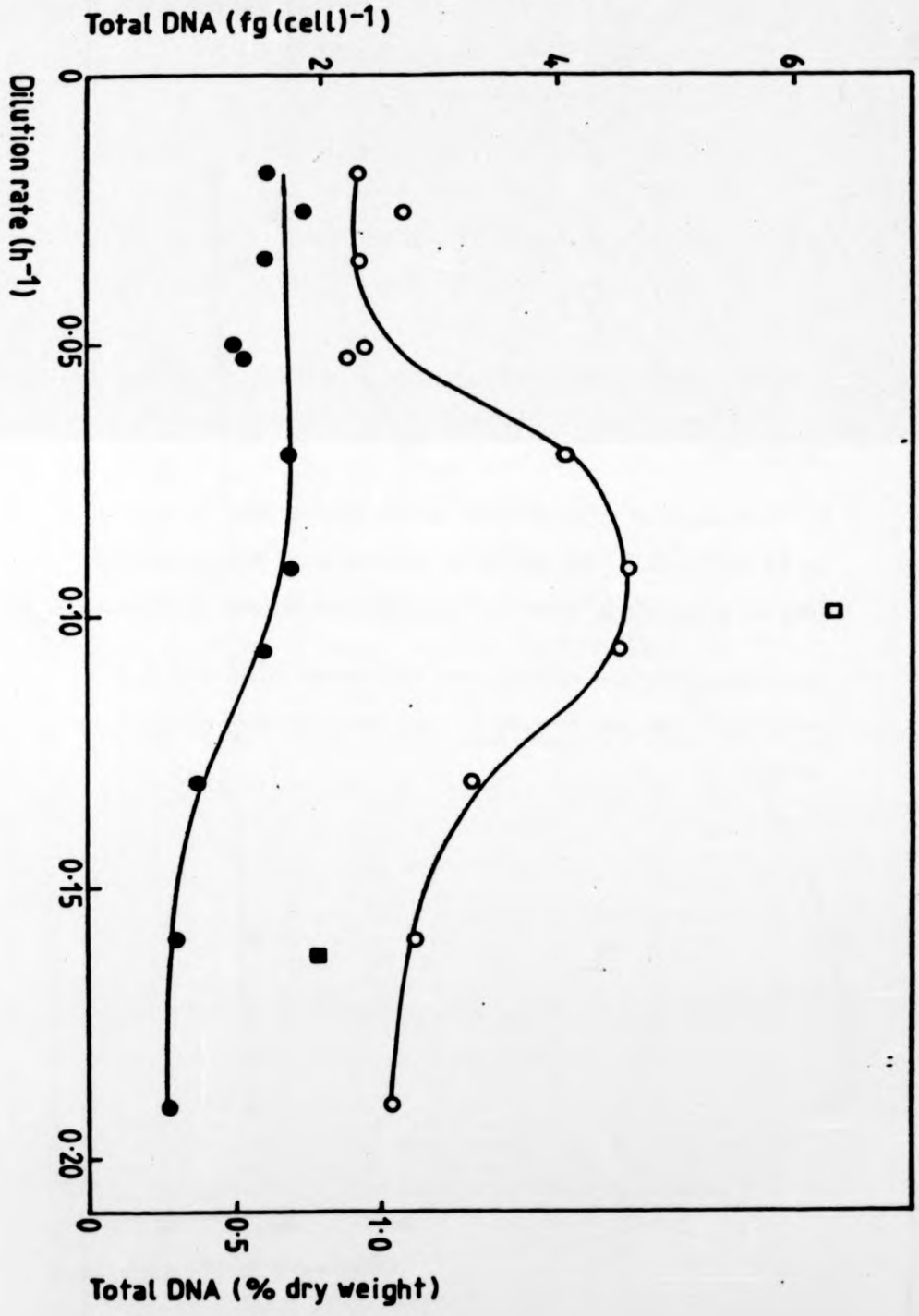
The RNA content per cell increased in a sigmoidal manner from approximately 20 to 53 fg RNA $(cell)^{-1}$ with increasing growth rate (figure 4.2.). The RNA content on a percentage dry weight basis increased slightly with increasing dilution rate although it may have been constant at about the 5% level (figure 4.2.).

The decrease in DNA content per cell with increasing growth rates above 0.1 h^{-1} was not expected from previous work on this organism (Mann and Carr, 1974). It was thought that the A. nidulans culture was possibly contaminated so that two different populations were present which had different levels of DNA and which were dominant at different growth rates. On the other hand, two sub-types of A. nidulans may even have been present which contained

Figure 4.1.

The influence of dilution rate on the DNA content, expressed as fg DNA $(cell)^{-1}$ (O) and as a percentage of the dry weight (●), of A. nidulans grown in light-limited chemostat culture.

The results for the shift-up and shift-down experiments, at D=0.162 h^{-1} (■) and D=0.100 h^{-1} (□) respectively, are also shown.


Total DNA (fg (cell)$^{-1}$)
2
4
6
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0
0·5
1·0
Total DNA (% dry weight)

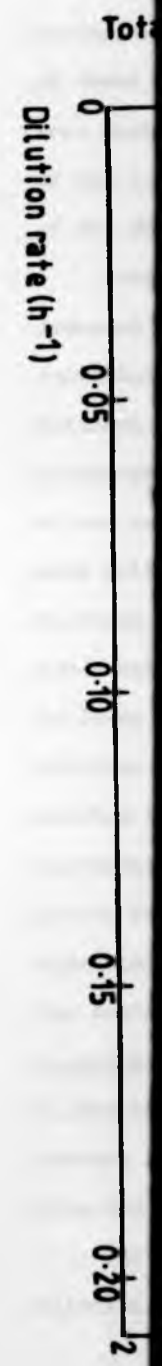
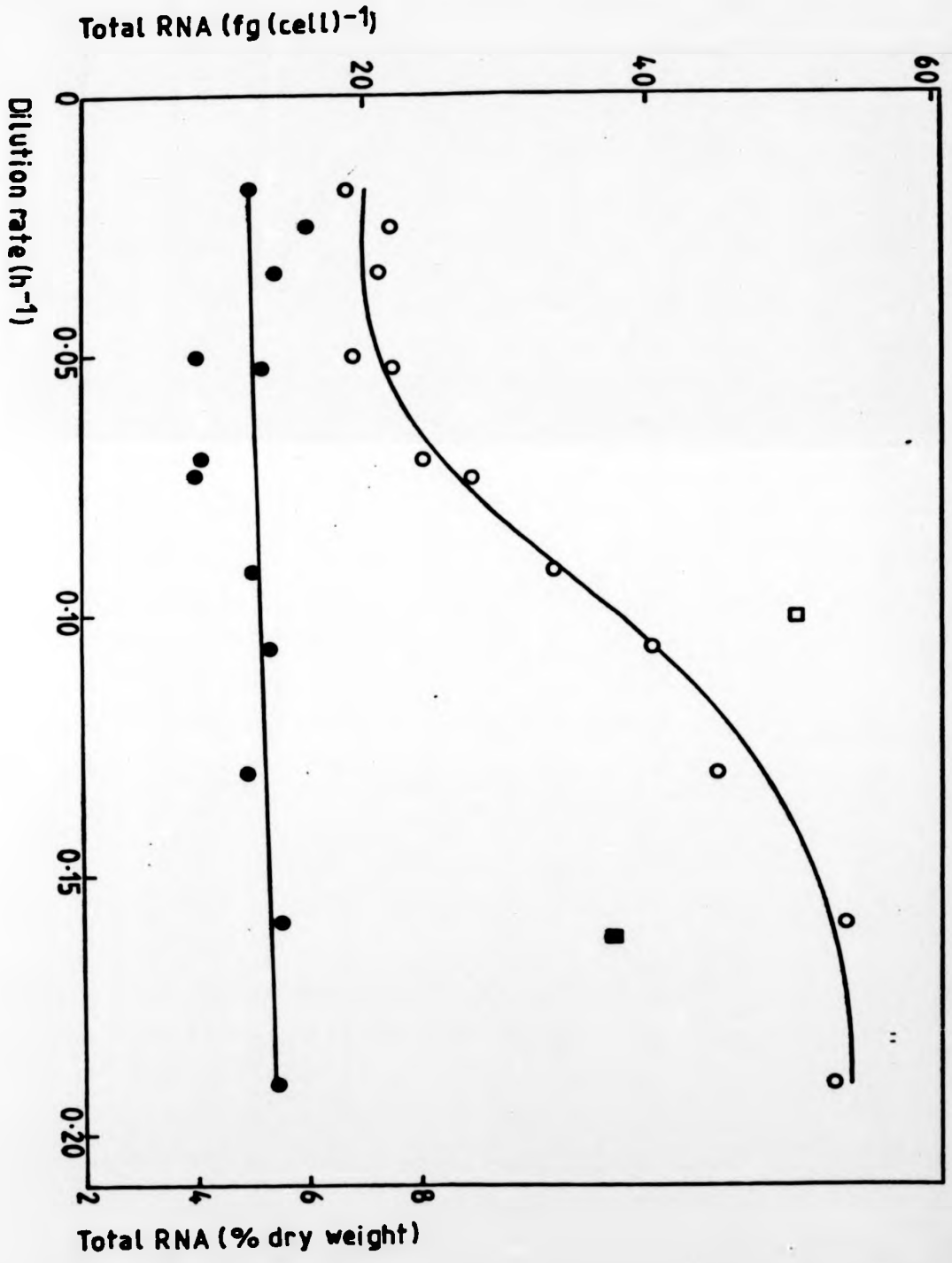


Figure 4.2.

The influence of dilution rate on the RNA content, expressed as fg RNA $(cell)^{-1}$ (○) and as a percentage of the dry weight (●), of A. nidulans grown in light-limited chemostat culture.

The results for the shift-up and shift-down experiments, at D=0.162 h^{-1} (■) and D=0.100 h^{-1} (□) respectively, are also shown.


Total RNA (fg (cell)$^{-1}$)
0
20
40
60
Dilution rate (h^{-1})
0·05
0·10
0·15
0·20
2
4
6
8
Total RNA (% dry weight)

different levels of DNA at different growth rates. In either of these cases if the cells with the higher levels of DNA were dominant at low growth rates and those with lower levels of DNA were dominant at higher growth rates then the pattern of DNA change would be expected to be the same as that obtained.

However, this phenomenon could not be explained by the presence of another organism, the culture having been checked for bacterial contamination at regular intervals by plating onto nutrient agar (section 2.3.) with contamination being negligible or non-existant. Consequently, the possibility of the presence of two sub-types of A. nidulans was determined by carrying out some shift-up and shift-down experiments (Maaløe and Kjeldgaard, 1966). In these experiments the organisms were left for approximately one-and-a-half generation times in a new dilution rate (either higher or lower respectively) so allowing DNA replication and cell division to occur but not allowing the organisms to become fully adapted to the new growth rate. It was assumed that if two different sub-types were present, the dominant type at the original growth rate would still be dominant after this time as the other sub-type would not have sufficient time to be selected and become the dominant member of the culture. On the other hand if the A. nidulans culture was really pure, as expected, and this change in DNA with growth rate was a physiological response then the changes in DNA content due to these shifts in growth rate would be expected to be much more rapid.

For the shift-down experiment the organism was grown at a dilution rate of 0.160 h^{-1} for a week to ensure that a steady

state was attained and that the 'low' DNA cells, if present, would be selected for. The dilution rate was reduced after this time to 0.100 h^{-1} and the system left for approximately one-and-a-half generation times at this rate. The cells were harvested and determinations carried out as previously (section 2.6.1.). A similar method was used for the shift-up experiment which was switched from a dilution rate of 0.098 to 0.162 h^{-1} to select for the 'high' DNA cells, if present.

The results from these experiments for DNA content did not indicate the presence of two sub-types as a high value of 6.4 fg DNA $(cell)^{-1}$ was obtained from the shift-down experiment and a low value of 2.0 fg DNA $(cell)^{-1}$ from the shift-up experiment (figure 4.1).

A value of 37.6 fg RNA $(cell)^{-1}$ was obtained for the RNA content from the shift-up experiment and 50.6 fg RNA $(cell)^{-1}$ from the shift-down experiment (figure 4.2.). These values were lower and higher respectively than would be expected for the corresponding steady state growth rates that the organisms were shifted to.

These experiments did not, therefore, explain the DNA results so a number of growth rates were repeated again and two growth rates, 0.129 and 0.190 h^{-1} were repeated and kept growing in steady states for a week to see if lack of a proper steady state was the reason for the low DNA values at these growth rates. However, in all cases, similar results were obtained as previously (figures 4.1. and 4.2.).

Some batch culture growth experiments were carried out to

determine whether similar results to Mann and Carr (1974) could be achieved. Different growth rates were obtained by altering the light intensity and the cells were harvested during the exponential log phase of growth when an absorbance level of between 0.4 and 0.6 had been reached. However, difficulties were encountered as the lowest reasonable growth rate which could be achieved under the culture conditions used was 0.118 h^{-1} and the highest rate was 0.200 h^{-1}. DNA and RNA determinations were carried out on organisms grown at these rates and the results did seem to differ slightly from the results obtained at similar growth rates in continuous culture. The DNA content at 0.200 h^{-1} was 4.2 fg DNA $(cell)^{-1}$ and RNA content was 61.5 fg RNA $(cell)^{-1}$ and at 0.118 h^{-1} the DNA content was 3.2 fg DNA $(cell)^{-1}$ and RNA content was 31.0 fg RNA $(cell)^{-1}$. In all cases the values were higher at 0.200 h^{-1} and lower at 0.118 h^{-1} than under continuous culture conditions.

From the results obtained at each growth rate under continuous culture conditions the specific rates of DNA and RNA synthesis were calculated, designated q_{DNA} and q_{RNA} respectively (section 2.6.1). The q_{DNA} values showed a linear increase up to a dilution rate just below 0.1 h^{-1} and above this dilution rate the rate of DNA synthesis was constant showing apparently no relation to growth rate (figure 4.3.). The q_{RNA} values showed a very different pattern with a linear increase with increasing dilution rate (figure 4.4.) indicating that RNA synthesis was proportional to the growth rate over the whole growth rate range studied.

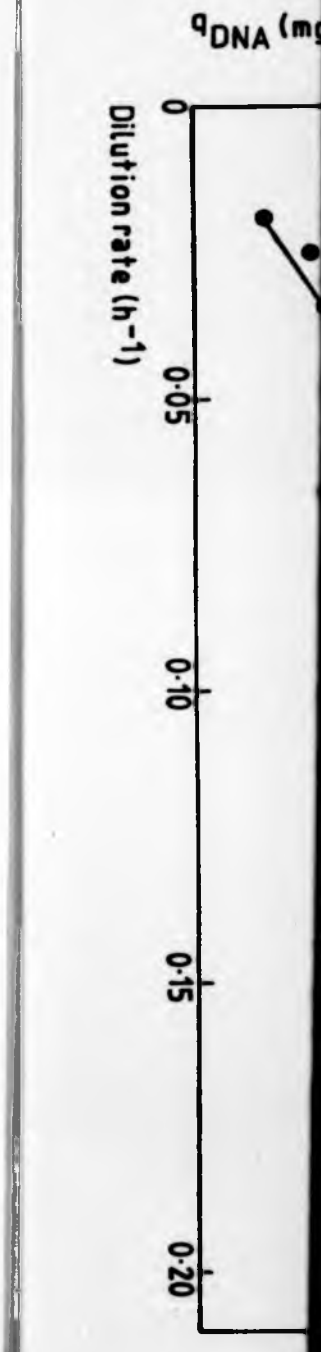
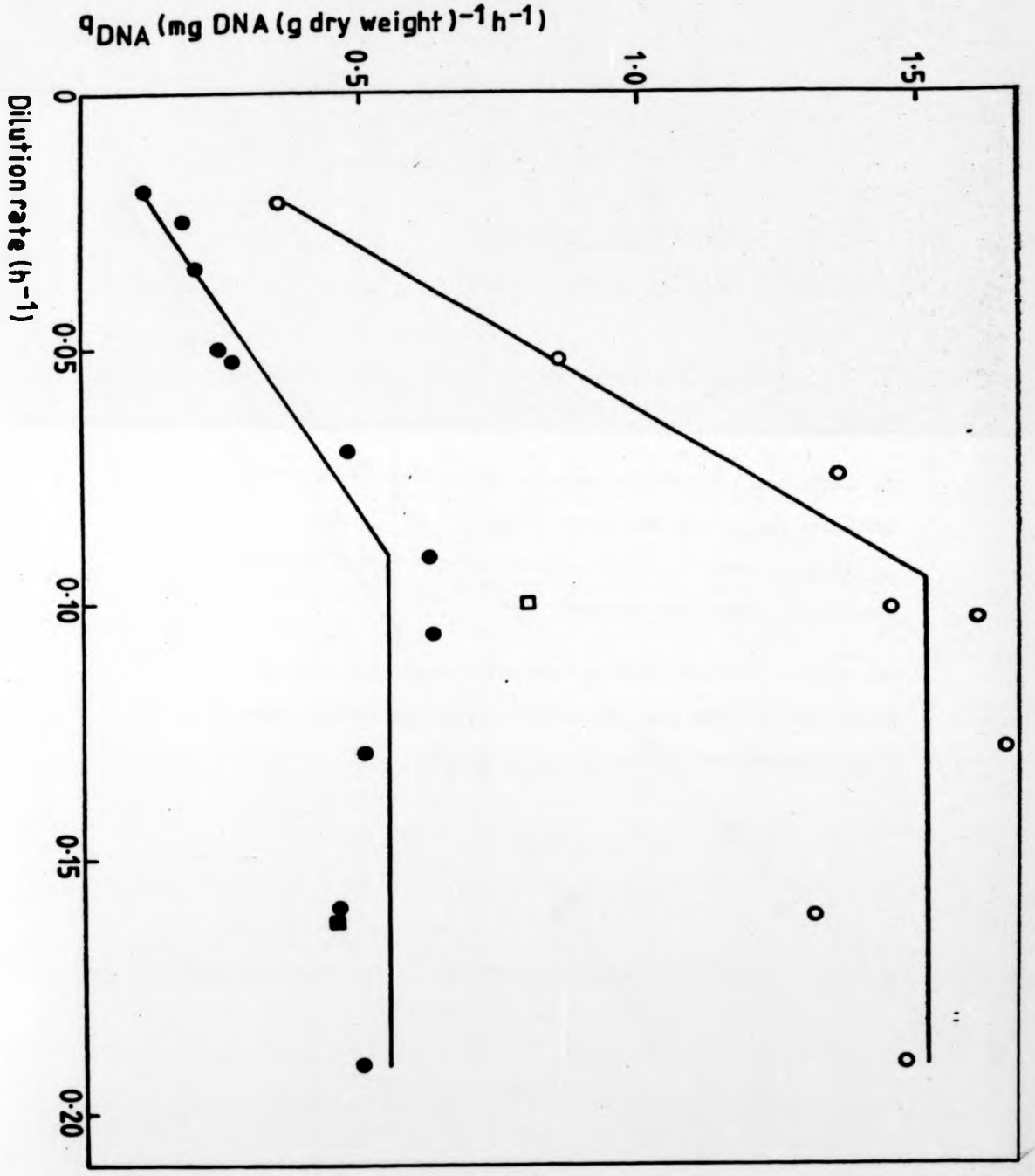


Figure 4.3.

The influence of dilution rate on the specific rate of DNA synthesis (q_{DNA}, mg DNA (g dry weight)$^{-1}$ h^{-1}) for *A. nidulans* grown in light-limited (●) and carbon dioxide-limited (○) chemostat culture.

The results for the shift-up and shift-down experiments, at D=0.162 h^{-1} (■) and D=0.100 h^{-1} (□) respectively under light-limited conditions, are also shown.


q_{DNA} (mg DNA (g dry weight)$^{-1}$ h^{-1})
0·5
1·0
1·5
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20

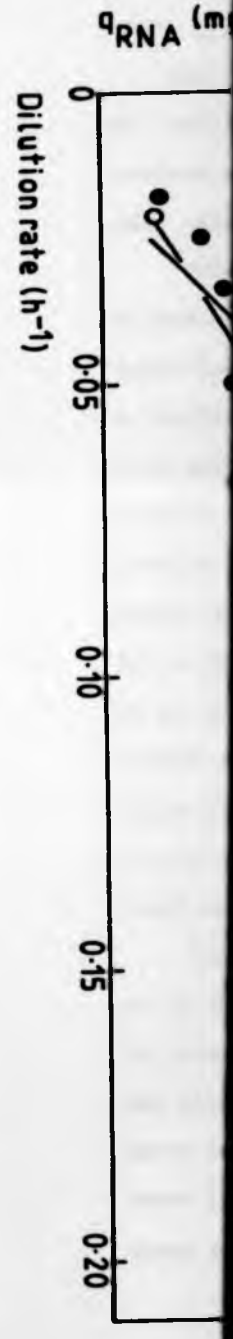
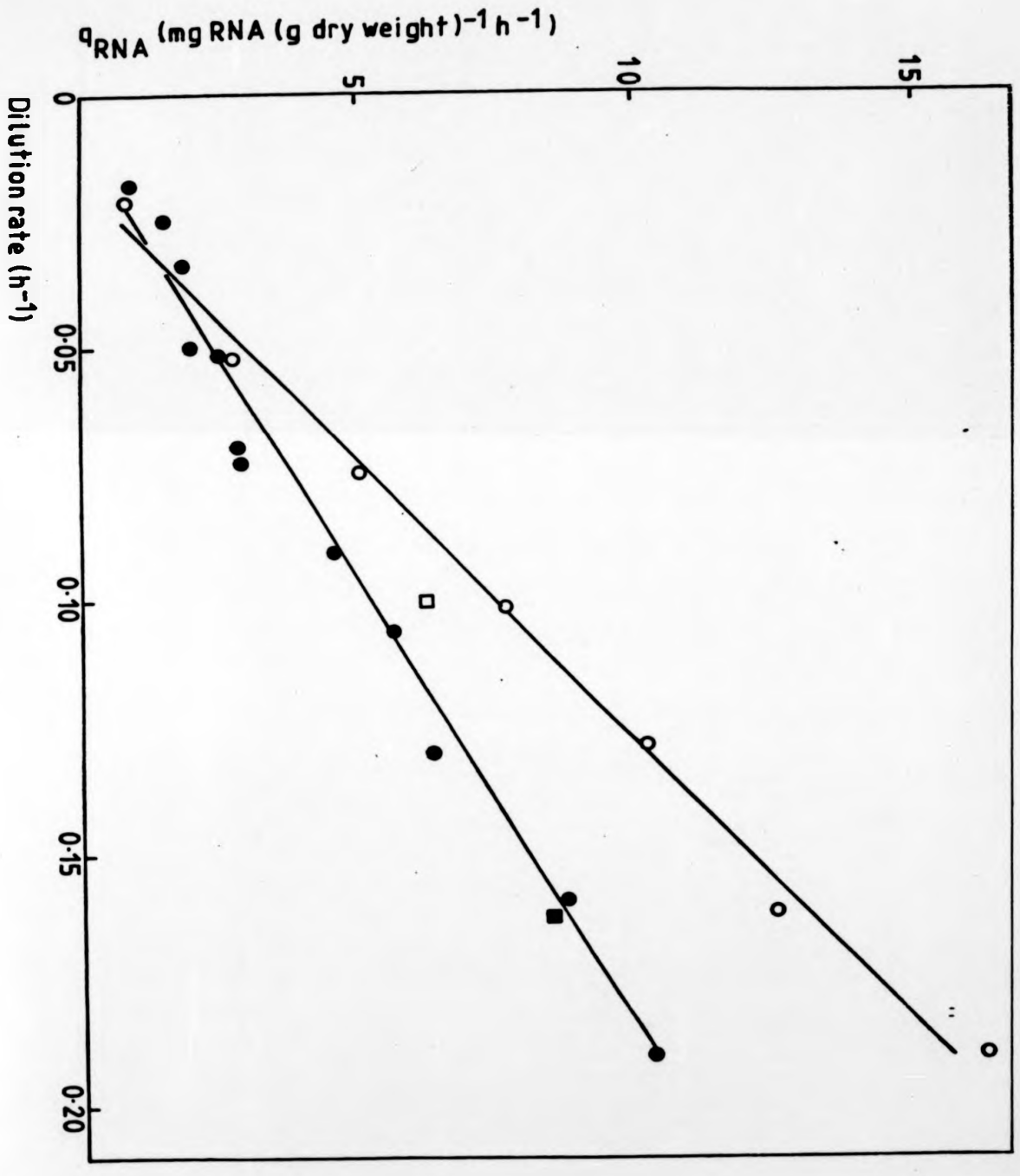


Figure 4.4.

The influence of dilution rate on the specific rate of RNA synthesis (q_{RNA}, mg RNA (g dry weight)$^{-1}$ h^{-1}) for *A. nidulans* grown in light-limited (●) and carbon dioxide-limited (○) chemostat culture.

The results for the shift-up and shift-down experiments, at D=0.162 h^{-1} (■) and D=0.100 h^{-1} (□) respectively under light-limited conditions, are also shown.


q_{RNA} (mg RNA (g dry weight)$^{-1}$ h^{-1})
0
5
10
15
Dilution rate (h^{-1})
0·05
0·10
0·15
0·20

4.1.2. Carbon dioxide-limited conditions

The DNA and RNA contents of A. nidulans, expressed as fg $(cell)^{-1}$ and as a percentage of the dry weight, were determined on a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The DNA content per cell under these growth conditions showed a much less pronounced peak than under light-limited conditions reaching a maximum level of 7.7 fg DNA $(cell)^{-1}$ at a dilution rate of approximately 0.08 h^{-1} and then decreasing with further increase in dilution rate (figure 4.5). The DNA content on a percentage dry weight basis showed a similar pattern to that shown under light limitation with a constant value up to a dilution rate of approximately 0.09 h^{-1} followed by an approximate two fold decline with increasing growth rate up to 0.19 h^{-1} (figure 4.5.). However, the percentage dry weight values were about twice those obtained for light-limited organisms, this being due to the smaller cell size of the carbon dioxide-limited organisms at equivalent growth rates and an increase in cell DNA content.

The RNA content per cell increased from approximately 20 to 53 fg RNA $(cell)^{-1}$ with increasing growth rate (figure 4.6.) as under light-limited conditions but the pattern of increase was slightly different with a sigmoidal increase not being shown in this case. The RNA content on a percentage dry weight basis increased significantly from 4 to 8% over the growth rate range examined (figure 4.6.) in contrast to the more or less

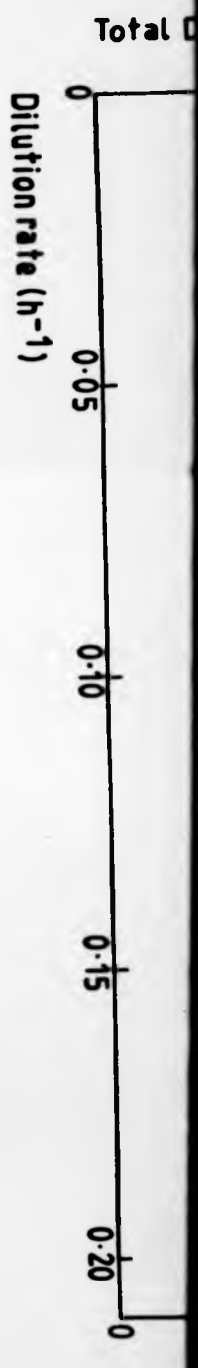
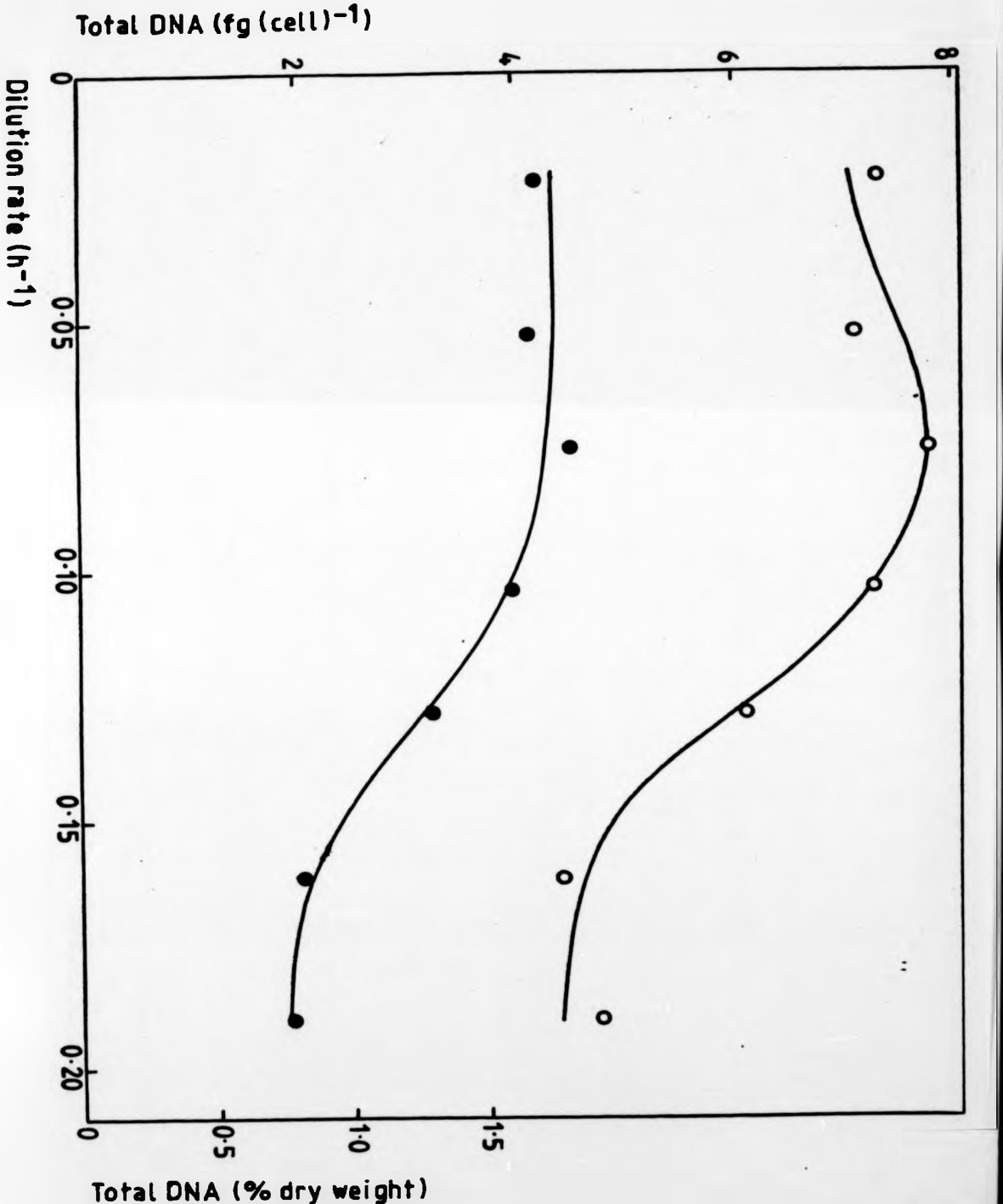


Figure 4.5.

The influence of dilution rate on the DNA content, expressed as fg DNA $(cell)^{-1}$ (○) and as a percentage of the dry weight (●), of A. nidulans grown in carbon dioxide-limited chemostat culture.


Total DNA (fg (cell)$^{-1}$)
0
2
4
6
8
Dilution rate (h^{-1})
0·05
0·10
0·15
0·20
0
0·5
1·0
1·5
Total DNA (% dry weight)

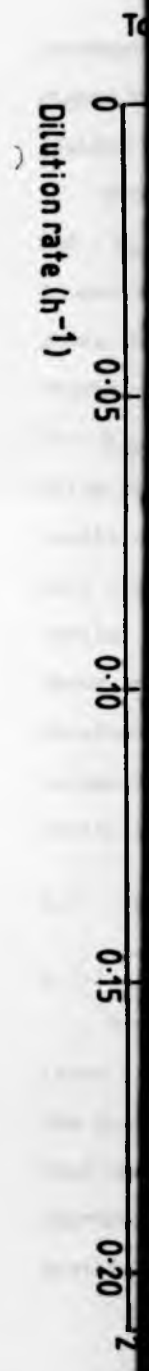
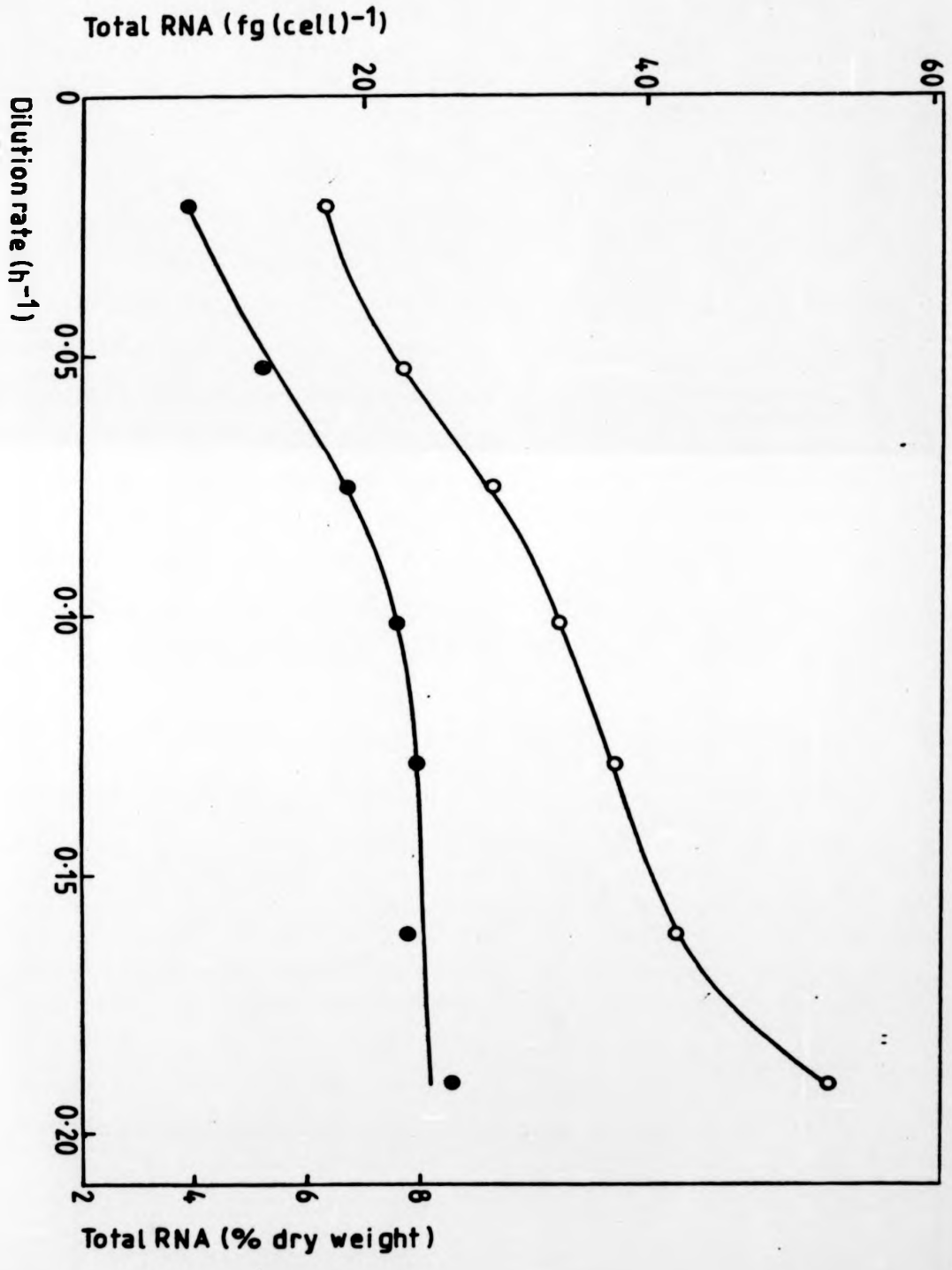


Figure 4.6.

The influence of dilution rate on the RNA content, expressed as fg RNA $(cell)^{-1}$ (O) and as a percentage of the dry weight (●), of A. nidulans grown in carbon dioxide-limited chemostat culture.


Total RNA (fg (cell)$^{-1}$)
20
40
60
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
2
4
6
8
Total RNA (% dry weight)

constant RNA level shown under light-limited conditions. The higher values obtained again being due to the relatively smaller cell size under these growth conditions.

From the results obtained at each growth rate the q_{DNA} and q_{RNA} values were calculated. The q_{DNA} values showed a linear increase up to a dilution rate just below 0.1 h^{-1} and above this dilution rate the rate of DNA synthesis was constant showing apparently no relation to growth rate (figure 4.3.). The q_{RNA} values showed a linear increase with increasing dilution rate (figure 4.4.) indicating, as under light-limited conditions, that RNA synthesis was proportional to the growth rate over the whole growth rate range studied. Although very similar patterns of synthesis rates for both DNA and RNA were shown under the two limitations, different actual values were obtained depending on the nature of the growth conditions at comparable growth rates, with the rates under carbon dioxide-limitation being greater than under light-limitation.

4.2. PROTEIN COMPOSITION

4.2.1. Pigment extraction

Removal of the cell pigments by the method of Vernon and Kamen (1953) (section 2.6.2.) had no significant effect on the protein values obtained. Consequently, it was presumed that the pigmentation of the cells did not interfere with the spectrophotometric assay method used. Consequently, the protein estimation was carried out on whole cells without

pigment extraction.

The only method in which significantly different results were obtained for protein estimation was when the cells were disrupted by two passages through the French pressure cell at 8.3×10^7 Pa at 4°C. The resulting cell extract obtained after centrifugation contained significantly less protein due presumably mainly to the loss of cell wall proteins.

4.2.2. Light-limited conditions

The protein content of A. nidulans, expressed as pg $(cell)^{-1}$ and as a percentage of the dry weight, was determined on a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The protein content per cell followed a very similar pattern to that shown by the DNA content under light-limited conditions reaching a maximum value of 0.33 pg protein $(cell)^{-1}$ at a dilution rate of 0.1 h^{-1} and then decreasing with further increase in dilution rate (figure 4.7). The protein content on a percentage dry weight basis again showed a similar pattern to that obtained for the DNA content being constant at about 43% up to a dilution rate of approximately 0.1 h^{-1} followed by an approximate two-fold decrease with increasing growth rate up to 0.19 h^{-1} (figure 4.7).

4.2.3. Carbon dioxide-limited conditions

The protein content of A. nidulans, expressed as pg $(cell)^{-1}$ and as a percentage of the dry weight, was determined on a

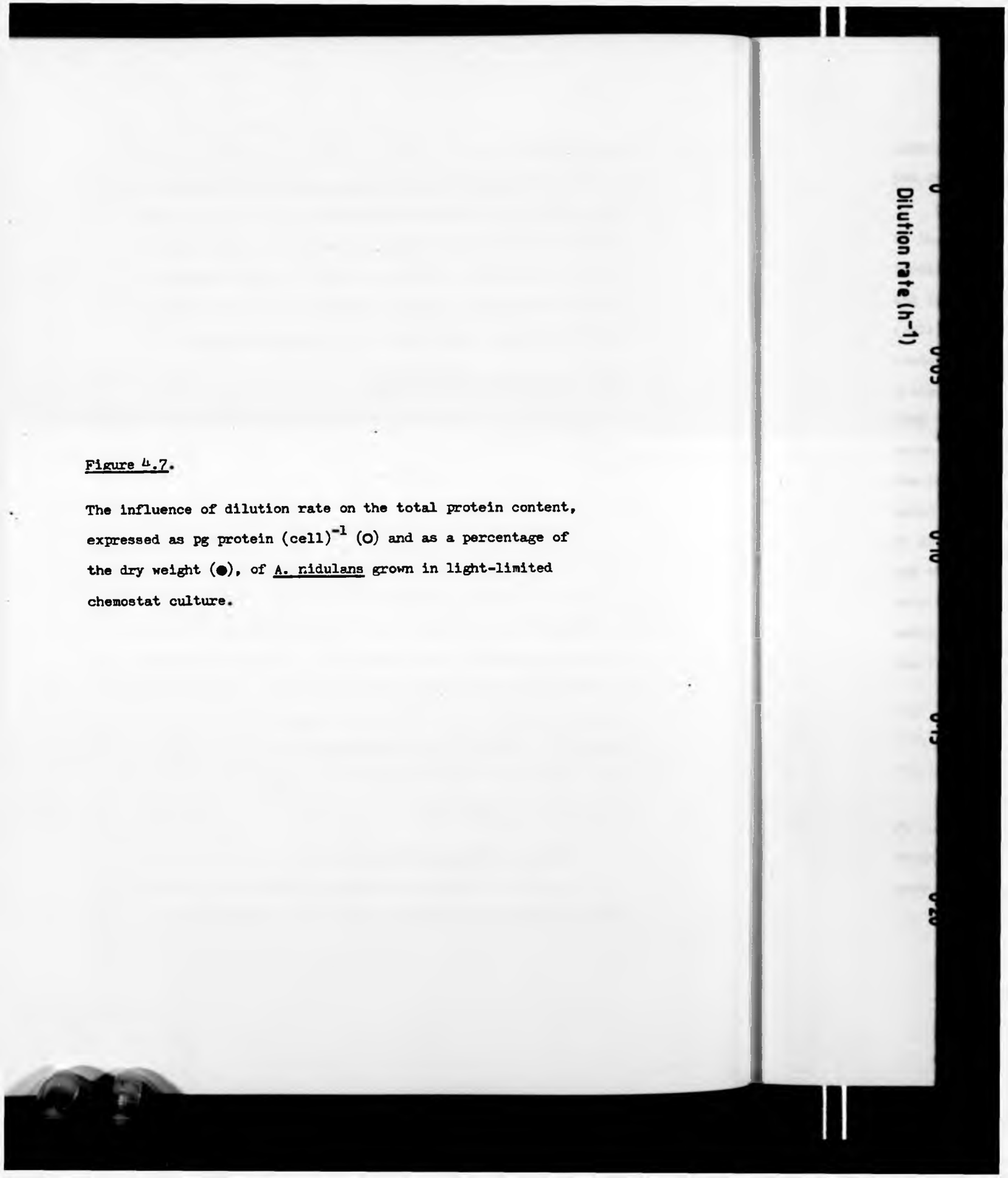
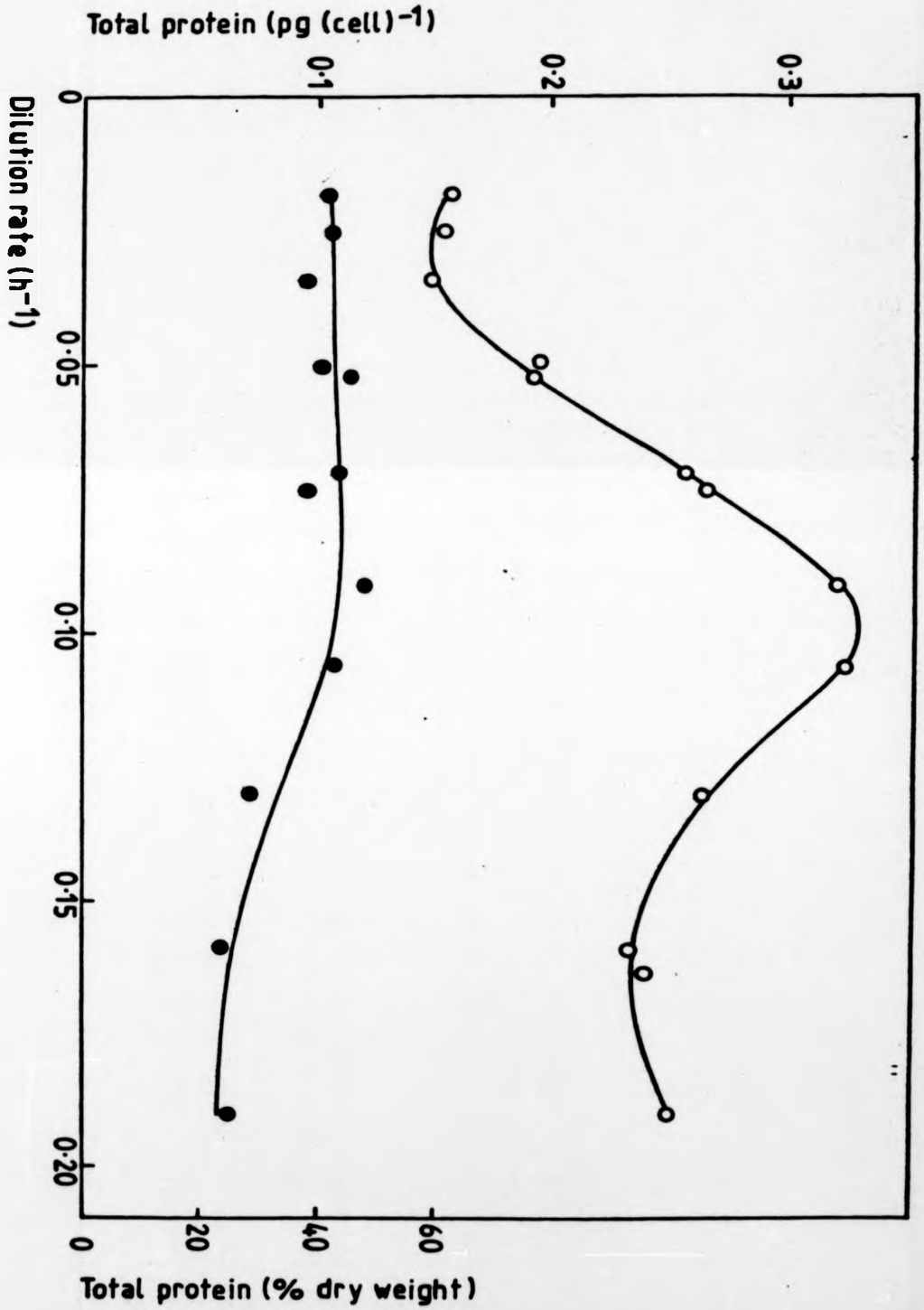


Figure 4.7.

The influence of dilution rate on the total protein content, expressed as pg protein $(cell)^{-1}$ (O) and as a percentage of the dry weight (●), of A. nidulans grown in light-limited chemostat culture.


Total protein (pg (cell)$^{-1}$)
0·1
0·2
0·3
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0
20
40
60
Total protein (% dry weight)

number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The protein content per cell showed a different pattern to that shown under light-limited conditions with a general increase with increasing growth rate at the low dilution rates and becoming more or less constant at about 0.25 pg protein $(cell)^{-1}$ at higher growth rates (figure 4.8.). The protein content on a percentage dry weight basis did, however, show a similar pattern to that obtained under light-limited conditions. With the exception of the value for the lowest dilution rate which regularly showed a lower protein content (presumably due to the pronounced limitation of carbon at this low growth rate) the percentage protein content was essentially constant at about 55% up to a dilution rate of approximately 0.13 h^{-1} and then decreased slightly with further increase in dilution rate (figure 4.8.). The percentage values obtained were again higher under carbon dioxide-limited conditions due to the relatively smaller cell size under these growth conditions.

4.3. PIGMENT COMPOSITION

4.3.1. Light-limited conditions

4.3.1.1. Chlorophyll a

The chlorophyll a content of A. nidulans, expressed as fg $(cell)^{-1}$ and as a percentage of the dry weight, was determined on a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The chlorophyll a content per cell increased with increasing

number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The protein content per cell showed a different pattern to that shown under light-limited conditions with a general increase with increasing growth rate at the low dilution rates and becoming more or less constant at about 0.25 pg protein $(cell)^{-1}$ at higher growth rates (figure 4.8.). The protein content on a percentage dry weight basis did, however, show a similar pattern to that obtained under light-limited conditions. With the exception of the value for the lowest dilution rate which regularly showed a lower protein content (presumably due to the pronounced limitation of carbon at this low growth rate) the percentage protein content was essentially constant at about 55% up to a dilution rate of approximately 0.13 h^{-1} and then decreased slightly with further increase in dilution rate (figure 4.8.). The percentage values obtained were again higher under carbon dioxide-limited conditions due to the relatively smaller cell size under these growth conditions.

4.3. PIGMENT COMPOSITION

4.3.1. Light-limited conditions

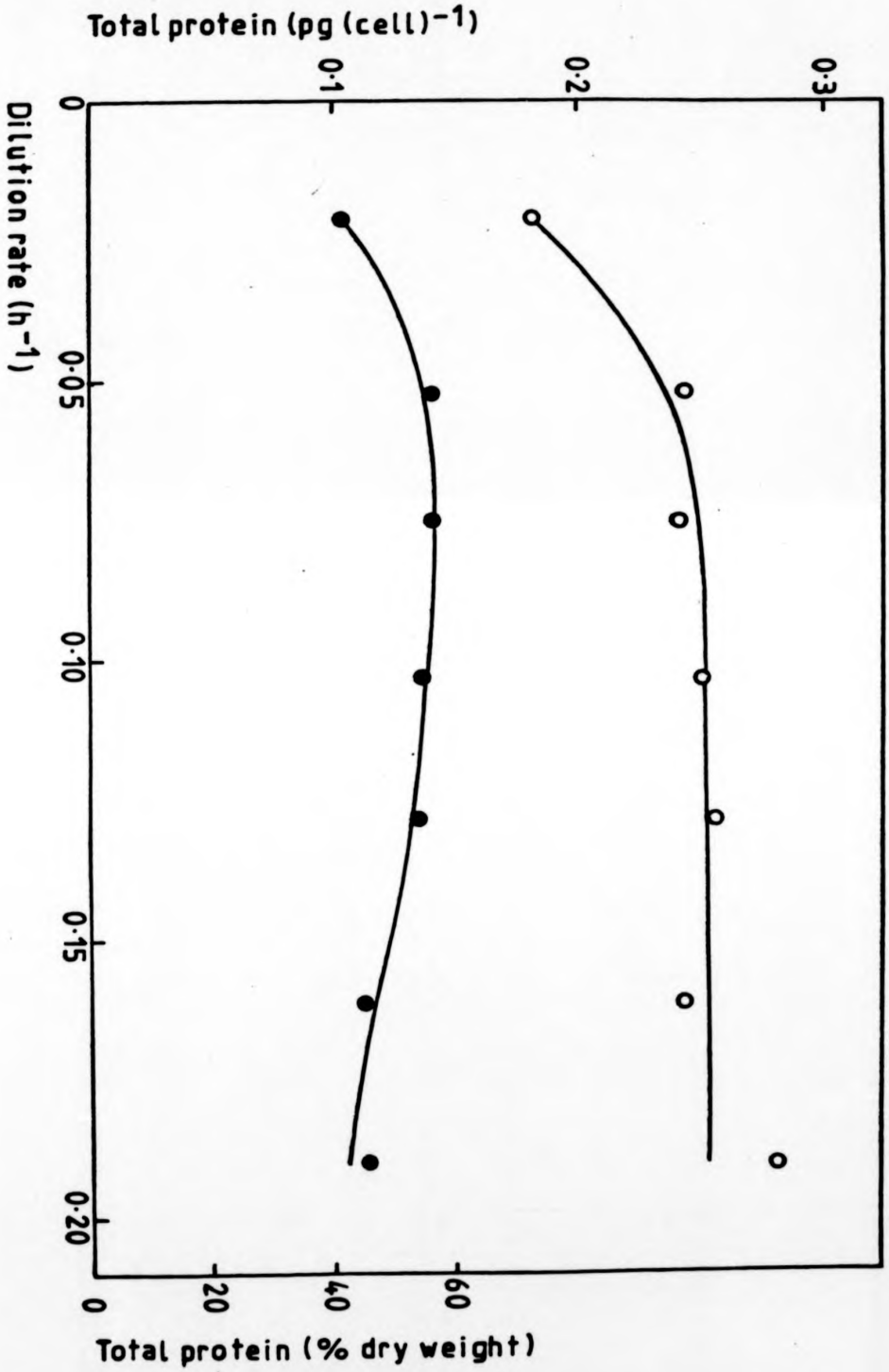
4.3.1.1. Chlorophyll a

The chlorophyll a content of A. nidulans, expressed as fg $(cell)^{-1}$ and as a percentage of the dry weight, was determined on a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The chlorophyll a content per cell increased with increasing

Figure 4.8.

The influence of dilution rate on the total protein content, expressed as pg protein $(cell)^{-1}$ (○) and as a percentage of the dry weight (●), of A. nidulans grown in carbon dioxide-limited chemostat culture.


Total protein (pg (cell)$^{-1}$)
0·1
0·2
0·3
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0
20
40
60
Total protein (% dry weight)

growth rate levelling off at the highest growth rates at a value of approximately 15 fg chlorophyll a(cell)$^{-1}$ (figure 4.9.). The chlorophyll a content on a percentage dry weight basis increased with increasing growth rate reaching a maximum of 2.1% at a dilution rate of approximately 0.075 h^{-1} and then decreased with further increases in the dilution rate (figure 4.9.).

4.3.1.2. Total carotenoids

The total carotenoid content of A. nidulans, expressed as fg (cell)$^{-1}$ and as a percentage of the dry weight, was determined on a number of steady state cultures over the dilution range, D=0.02 to 0.19 h^{-1}.

The total carotenoid content per cell increased with increasing growth rate over the whole growth rate range reaching a maximum of 7.3 fg total carotenoid (cell)$^{-1}$ at a dilution rate of 0.19 h^{-1} (figure 4.10). The total carotenoid content on a percentage dry weight basis tended to decrease slightly, from approximately 0.8 to 0.6%, with increasing growth rate (figure 4.10).

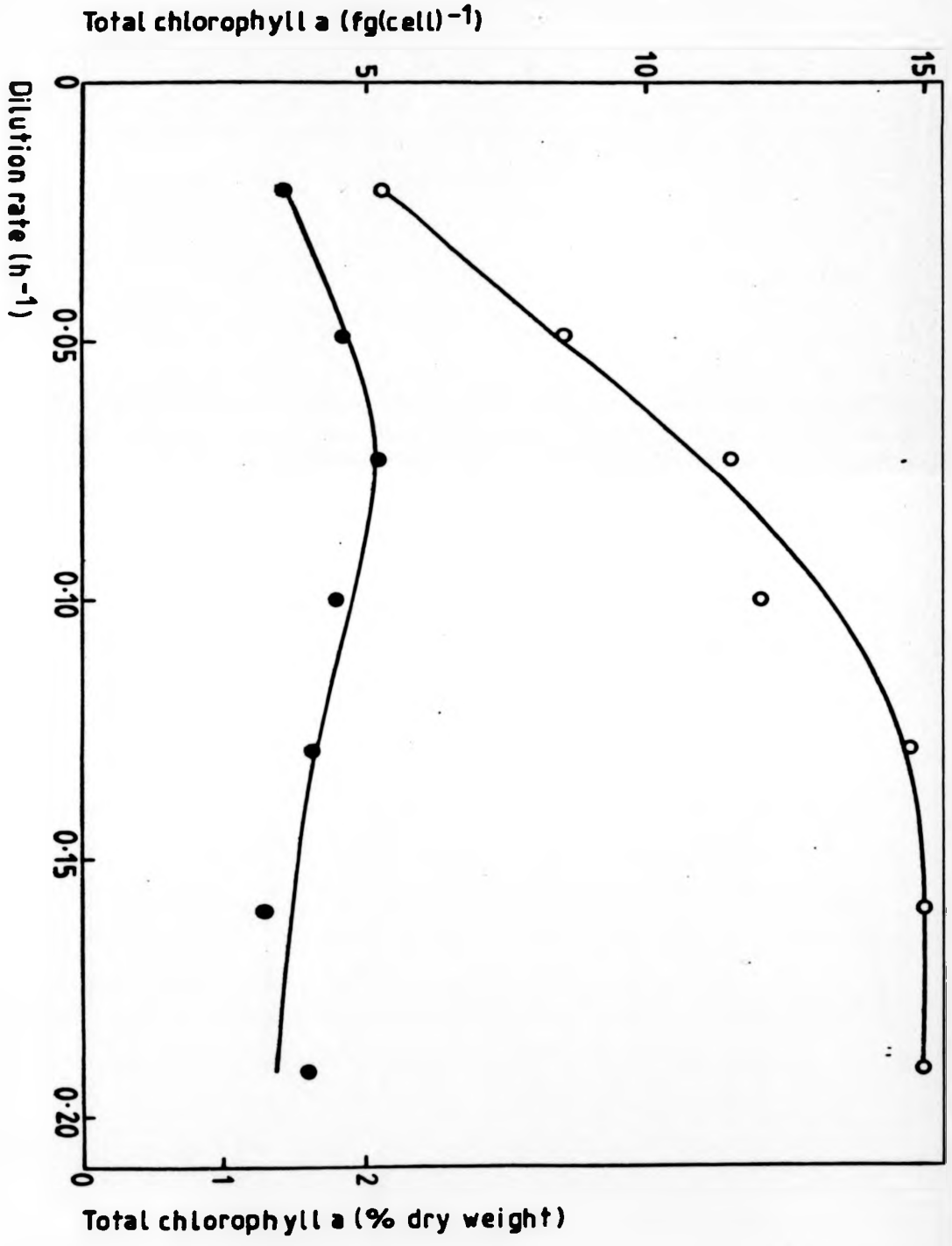
4.3.1.3. Phycocyanin

The phycocyanin content of A. nidulans, expressed as pg (cell)$^{-1}$ and as a percentage of the dry weight, was determined on a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The results obtained for the phycocyanin content, under these and carbon dioxide-limiting conditions, from the aqueous extract after breakage of the cells using the French pressure cell and from the aqueous extract after acetone extraction

Figure 4.9.

The influence of dilution rate on the chlorophyll a content, expressed as fg chlorophyll a(cell)$^{-1}$ (○) and as a percentage of the dry weight (●), of A. nidulans grown in light-limited chemostat culture.


Total chlorophyll a (fg(cell)$^{-1}$)
0
5
10
15
Dilution rate (h^{-1})
0·05
0·10
0·15
0·20
0
1
2
Total chlorophyll a (% dry weight)

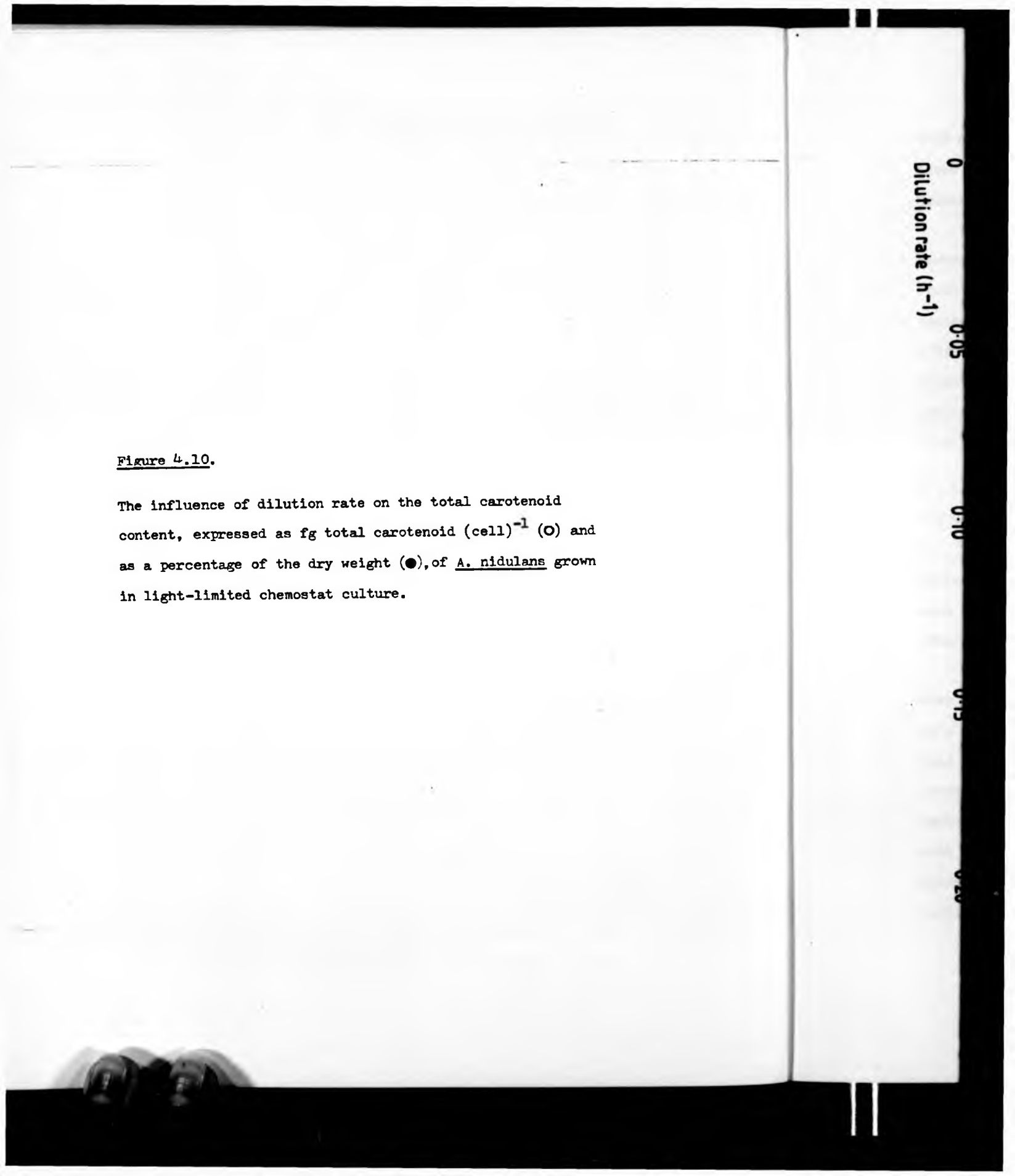
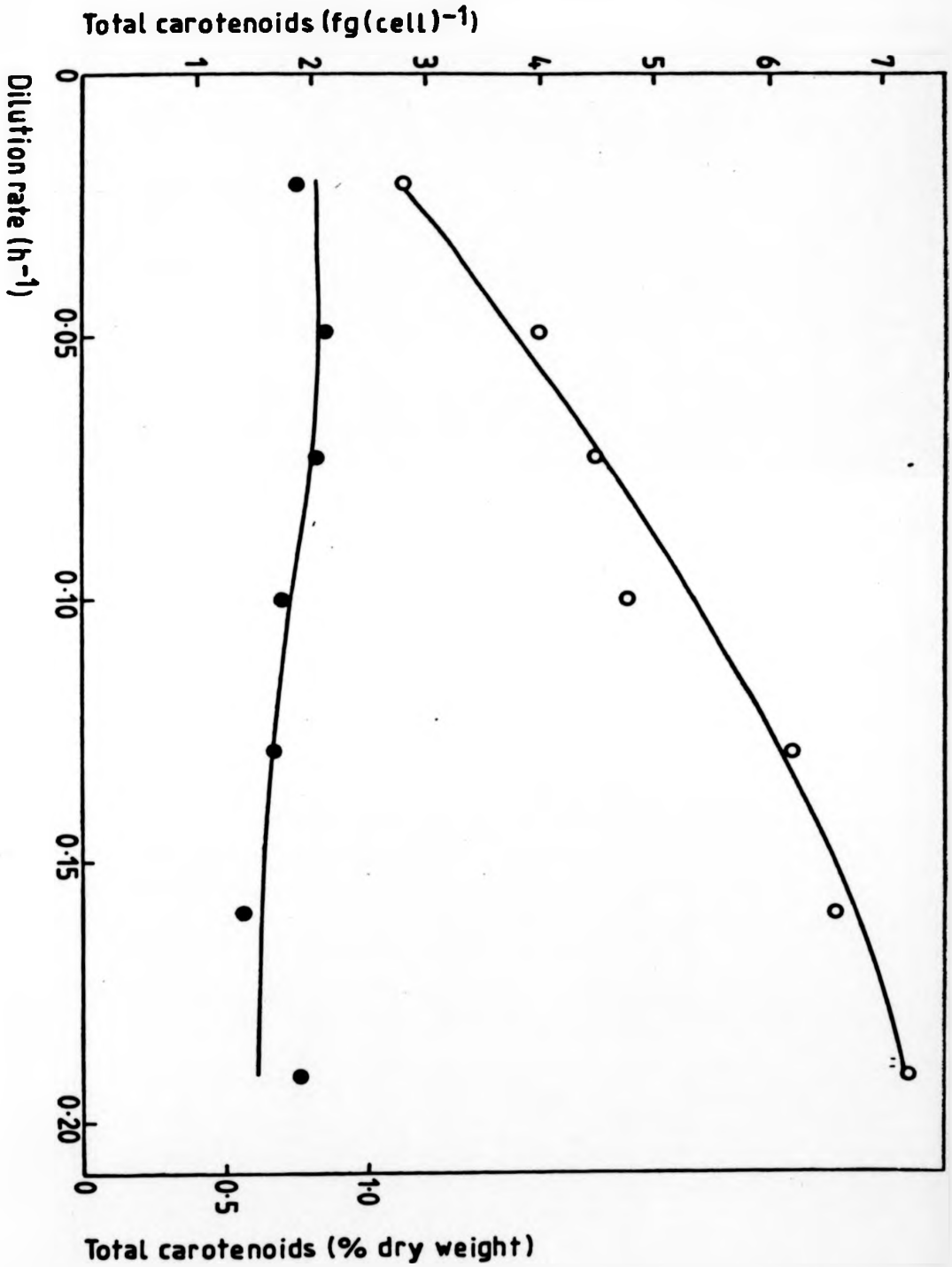


Figure 4.10.

The influence of dilution rate on the total carotenoid content, expressed as fg total carotenoid $(cell)^{-1}$ (O) and as a percentage of the dry weight (●), of *A. nidulans* grown in light-limited chemostat culture.


Total carotenoids (fg(cell)$^{-1}$)
1
2
3
4
5
6
7
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0
0·5
1·0
Total carotenoids (% dry weight)

were not significantly different. The results given are those from the former method as the latter method was used primarily as a check of the results obtained.

The phycocyanin content per cell increased with increasing growth rate up to a maximum of 0.104 pg phycocyanin $(cell)^{-1}$ at a dilution rate of approximately 0.13 h^{-1} and then decreased slightly with further increase in dilution rate (figure 4.11.). The phycocyanin content on a percentage dry weight basis reached a maximum value of 14.6% at a dilution rate of approximately 0.07 h^{-1} and then decreased with further increases in dilution rate (figure 4.11.).

4.3.2. Carbon dioxide-limited conditions

4.3.2.1. Chlorophyll a

The chlorophyll a content of A. nidulans, expressed as fg $(cell)^{-1}$ and as a percentage of the dry weight, was determined on a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The chlorophyll a content per cell showed a similar increase with increasing growth rate as under light-limited conditions to a maximum value of 15 fg chlorophyll a$(cell)^{-1}$ at a dilution rate of 0.16 h^{-1} but then decreased slightly with a further increase in the dilution rate (figure 4.12.). The chlorophyll a content on a percentage dry weight basis showed a general increase with increasing growth rate levelling off at the highest growth rates at a maximum value of approximately 2.3 fg chlorophyll a $(cell)^{-1}$ (figure 4.12) in contrast to the decrease in chlorophyll a

Dilution rate (h^{-1})

Figure 4.11.

The influence of dilution rate on the phycocyanin content, expressed as pg phycocyanin $(cell)^{-1}$ (O) and as a percentage of the dry weight (●), of A. nidulans grown in light-limited chemostat culture.

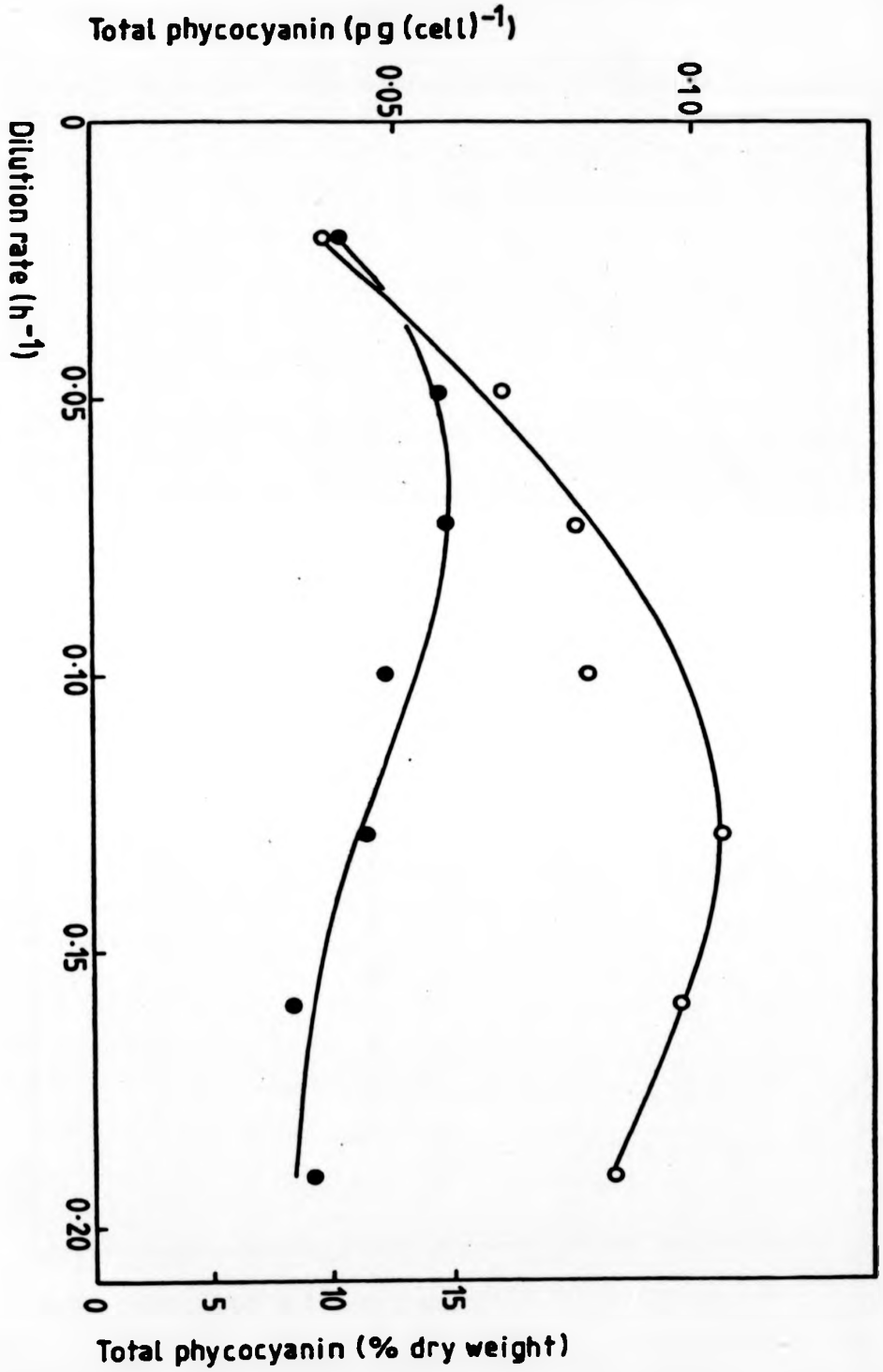
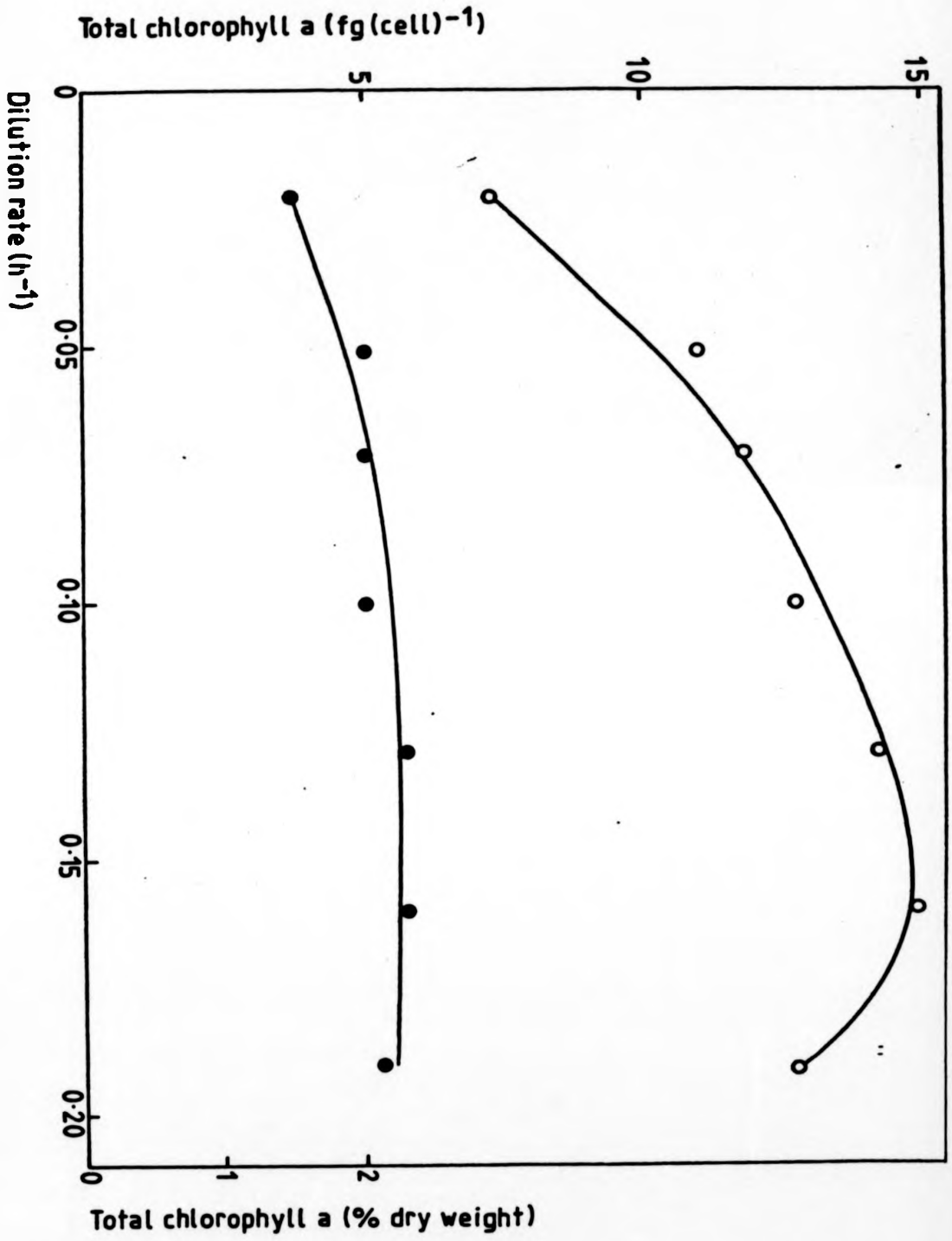

Total phycocyanin (pg (cell)$^{-1}$)
0·05
0·10
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0
5
10
15
Total phycocyanin (% dry weight)

Figure 4.12.

The influence of dilution rate on the chlorophyll a content, expressed as fg chlorophyll a $(cell)^{-1}$ (○) and as a percentage of the dry weight (●), of A. nidulans grown in carbon dioxide-limited chemostat culture.


Total chlorophyll a (fg (cell)$^{-1}$)
0
5
10
15
Dilution rate (h^{-1})
0·05
0·10
0·15
0·20
0
1
2
Total chlorophyll a (% dry weight)

shown at high growth rates under light-limited conditions.

4.3.2.2. Total carotenoids

The total carotenoid content of A. nidulans, expressed as fg $(cell)^{-1}$ and as a percentage of the dry weight, was determined on a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The total carotenoid content per cell showed a very different pattern to that shown under light-limitation. The values were high at low growth rates and then decreased as the dilution rate increased to about 0.07 h^{-1}. This was followed by an increase with further increase in growth rate (figure 4.13.). The total carotenoid content on a percentage dry weight basis showed a similar trend to that shown on a per cell basis (figure 4.13.) which was again very different to that shown under light-limiting conditions.

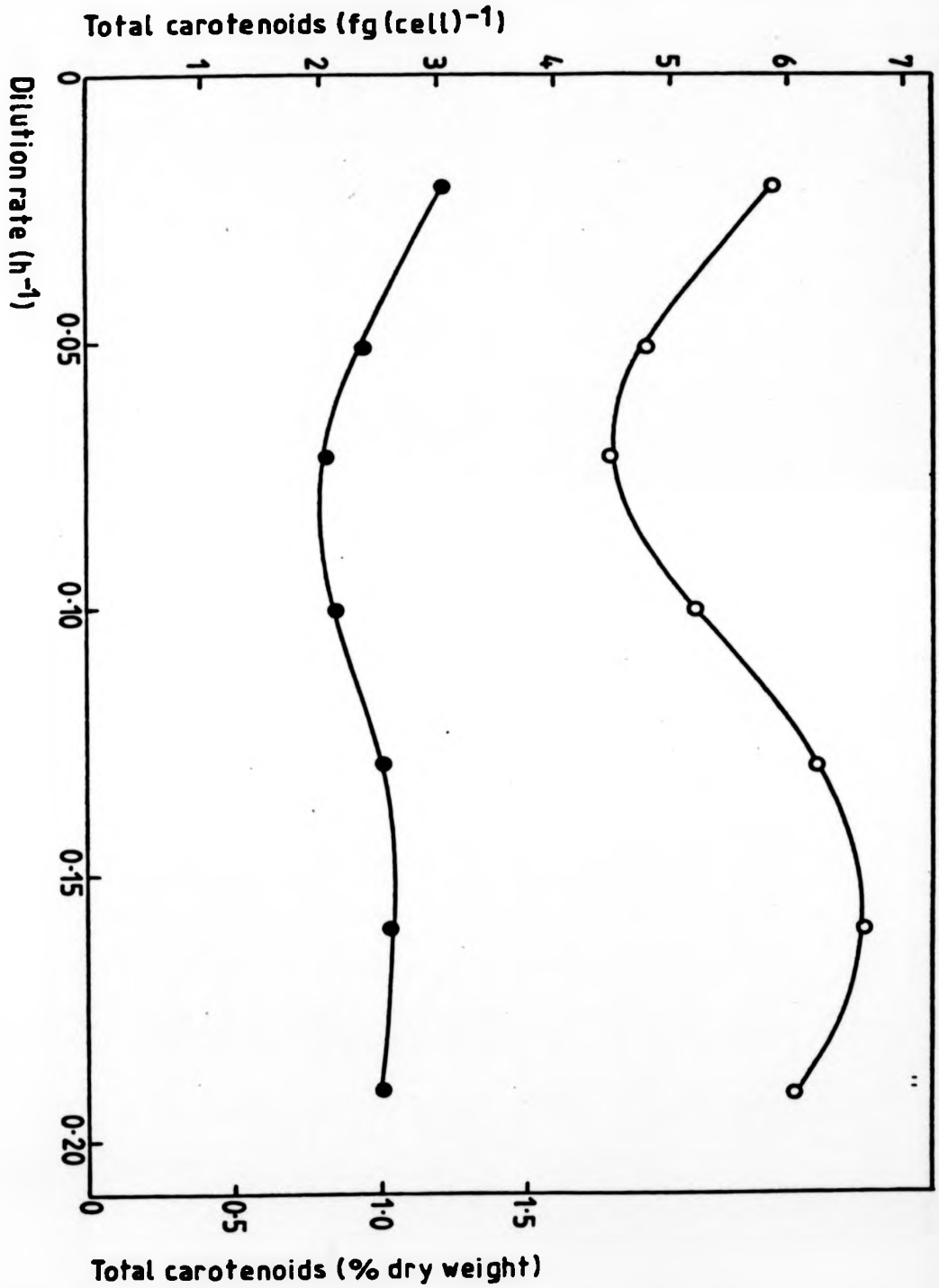
4.3.2.3. Phycocyanin

The phycocyanin content of A. nidulans, expressed as pg $(cell)^{-1}$ and as a percentage of the dry weight, was determined on a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The phycocyanin content per cell showed a similar pattern to that seen under light-limiting conditions with an increase with increasing growth rate up to a maximum of 0.096 pg phycocyanin $(cell)^{-1}$ at a dilution rate of approximately 0.13 h^{-1} followed by a decrease with further increase in dilution rate

Figure 4.13.

The influence of dilution rate on the total carotenoid content, expressed as fg total carotenoid $(cell)^{-1}$ (O) and as a percentage of the dry weight (●), of A. nidulans grown in carbon dioxide-limited chemostat culture.


Total carotenoids (fg (cell)$^{-1}$)
1
2
3
4
5
6
7
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0
0·5
1·0
1·5
Total carotenoids (% dry weight)

(figure 4.14.). The phycocyanin content on a percentage dry weight basis showed a similar trend to that shown on a per cell basis increasing with increasing growth rate up to a value of 16.5% at a dilution rate of approximately 0.1 h^{-1} followed by a decrease with further increase in dilution rate (figure 4.14.).

4.3.3. Pigment ratios

From the preceeding results of the pigment composition of A. nidulans the ratios of the pigments were calculated for organisms grown under both limitations (tables 4.1. and 4.2.).

4.3.3.1. Light-limited conditions

Despite large variations in the absolute values of the pigments and in the total percentage of pigment on a dry weight basis obtained at different growth rates there was very little variation in the phycocyanin /chlorophyll ratio or in the total carotenoid/chlorophyll ratio with changing growth rate(table 4.1.). There was a slight decrease in the phycocyanin/chlorophyll ratio between about 8 to 6 over the growth rate range studied (figure 4.15.) and even less variation in the total carotenoid/ chlorophyll ratio with values around 0.4 to 0.5.

4.3.3.2. Carbon dioxide-limited conditions

There was little variation in the phycocyanin/chlorophyll ratio or in the total carotenoid/chlorophyll ratio with changing growth rate, except for the lowest growth rates (table 4.2.).

Dilution rate (h^{-1})

Figure 4.14.

The influence of dilution rate on the phycocyanin content, expressed as pg phycocyanin $(cell)^{-1}$ (○) and as a percentage of the dry weight (●), of *A. nidulans* grown in carbon dioxide-limited chemostat culture.

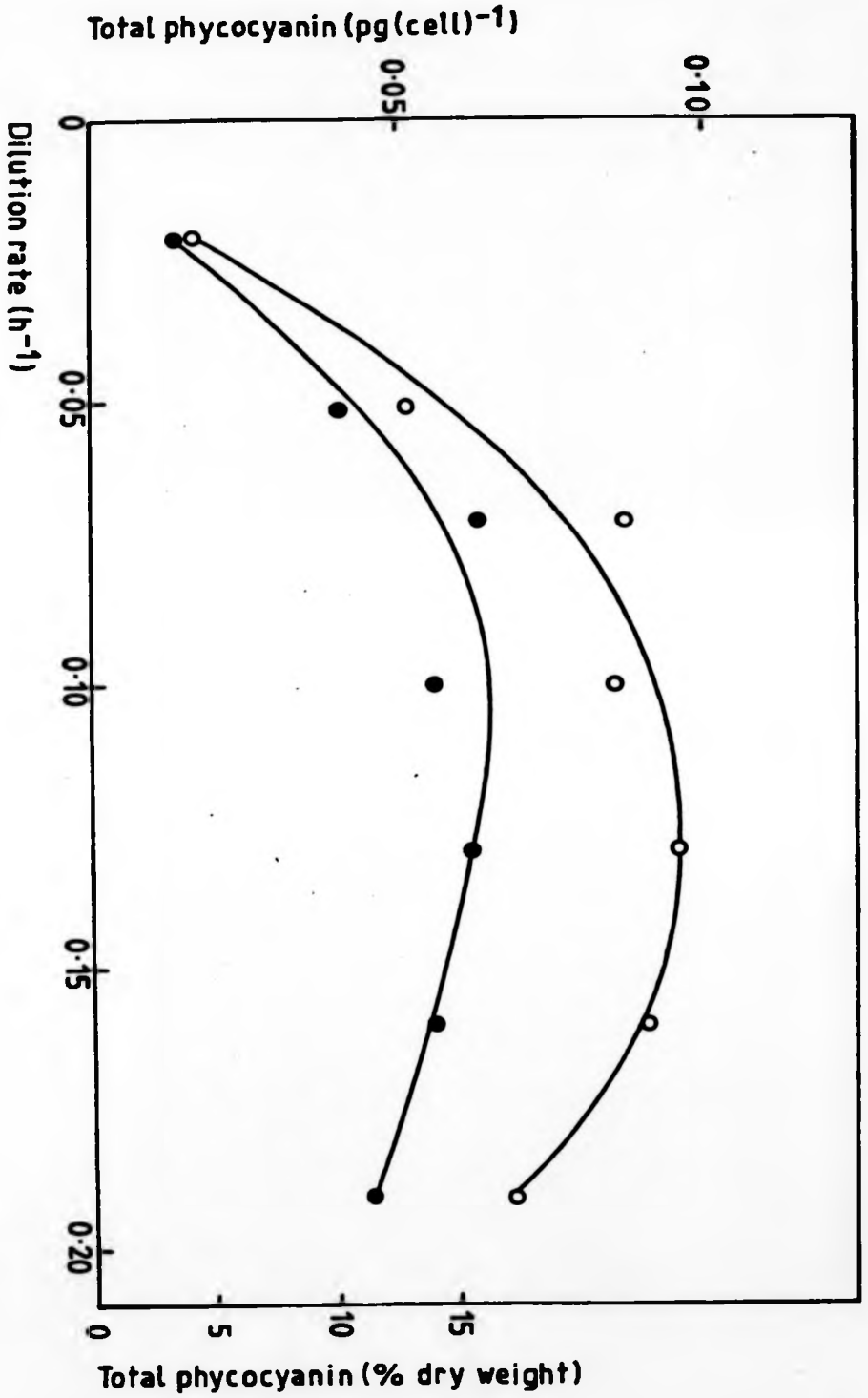

Total phycocyanin (pg(cell)$^{-1}$)
0·05
0·10
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0
5
10
15
Total phycocyanin (% dry weight)

TABLE 4.1. The influence of dilution rate on the pigment ratios for A. nidulans grown in light-limited chemostat culture

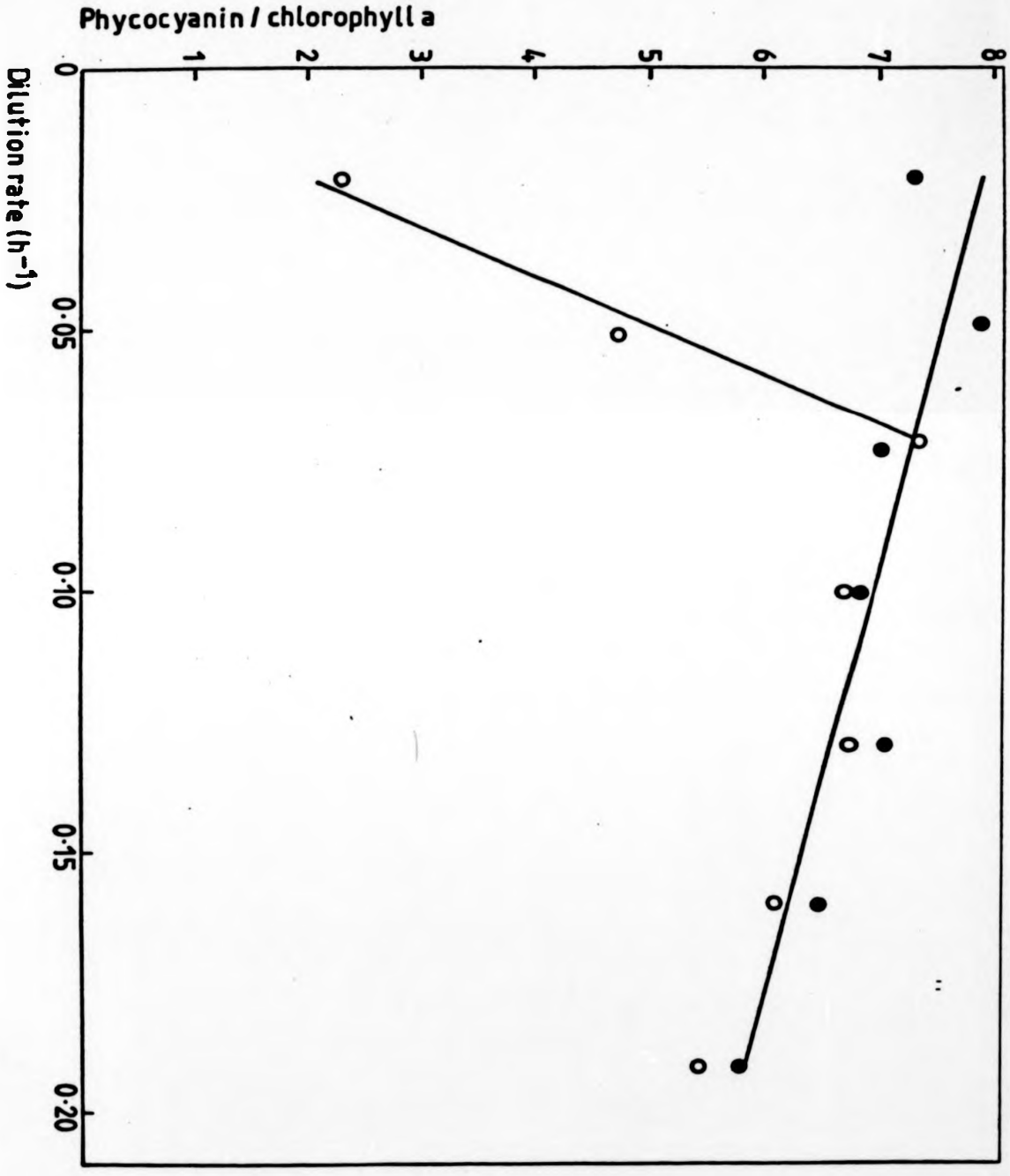
Dilution rate (h^{-1})	Chlorophyll : Total carotenoid : Phycocyanin	Total pigment (% dry weight)
0.021	1 : 0.53 : 7.31	12.54
0.049	1 : 0.47 : 7.88	17.47
0.073	1 : 0.39 : 7.00	17.50
0.100	1 : 0.40 : 6.83	13.75
0.129	1 : 0.42 : 7.04	13.69
0.160	1 : 0.44 : 6.49	10.15
0.191	1 : 0.48 : 5.81	11.56

TABLE 4.2. The influence of dilution rate on the pigment ratios for A. nidulans grown in carbon dioxide-limited chemostat culture

Dilution rate (h^{-1})	Chlorophyll : Total carotenoid : Phycocyanin	Total pigment (% dry weight)
0.021	1 : 0.80 : 2.28	8.91
0.051	1 : 0.44 : 4.68	14.29
0.071	1 : 0.38 : 7.34	20.37
0.100	1 : 0.41 : 6.73	18.36
0.129	1 : 0.44 : 6.75	20.35
0.160	1 : 0.45 : 6.10	19.27
0.191	1 : 0.47 : 5.43	16.06

Figure 4.15.

The influence of dilution rate on the phycocyanin/chlorophyll a ratio for A. nidulans grown in light-limited (●) and carbon dioxide-limited (O) chemostat culture.


Phycocyanin / chlorophyll a
0
1
2
3
4
5
6
7
8
Dilution rate (h^{-1})
0·05
0·10
0·15
0·20

Very similar values for the phycocyanin/chlorophyll ratios were obtained as under light-limitation except for extremely low values at the lowest growth rates (figure 4.15). Also similar values to those of light-limited cultures of around 0.4 to 0.5 were obtained for the carotenoid/chlorophyll ratios except at the lowest growth rate of 0.02 h^{-1} where the ratio was approximately doubled.

4.4. CARBOHYDRATE COMPOSITION

4.4.1. Evaluation of methods

The results presented are those obtained using the phenol method (section 2.6.4.2.) as this proved to be more reliable than the anthrone method (section 2.6.4.1.), the resulting colours being more stable. This was especially evident at the very low carbohydrate concentrations obtained under carbon dioxide-limited growth conditions. Also, the results obtained using the anthrone method were in all cases slightly lower than those obtained using the phenol method as, for example, shown in figure 4.16. This was, in fact, expected (Herbert, Phipps and Strange, 1971) as the anthrone method approximates to a 'total hexose' method giving identical colours (though not of equal intensity) with all hexoses while other sugars give different and much weaker colours, whereas the phenol method approximates to a 'universal reagent' for all types of sugars, including the carbohydrate residues of nucleic acid.

4.4.2. Light-limited conditions

The carbohydrate content of A. nidulans, expressed as pg $(cell)^{-1}$ and as a percentage of the dry weight, was determined on a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The carbohydrate content per cell increased with increasing growth rate reaching a maximum of approximately 0.27 pg carbohydrate $(cell)^{-1}$ at a dilution rate of 0.12 h^{-1} and then decreased with further increase in dilution rate (figure 4.16.). A similar pattern was obtained using the anthrone method although the actual values were slightly lower and the maximum value was obtained closer to 0.1 h^{-1}. The carbohydrate content on a percentage dry weight basis increased up to a maximum value of 47% at a dilution rate of approximately 0.06 h^{-1} followed by a decrease with further increases in dilution rate (figure 4.16.).

4.4.3. Carbon dioxide-limited conditions

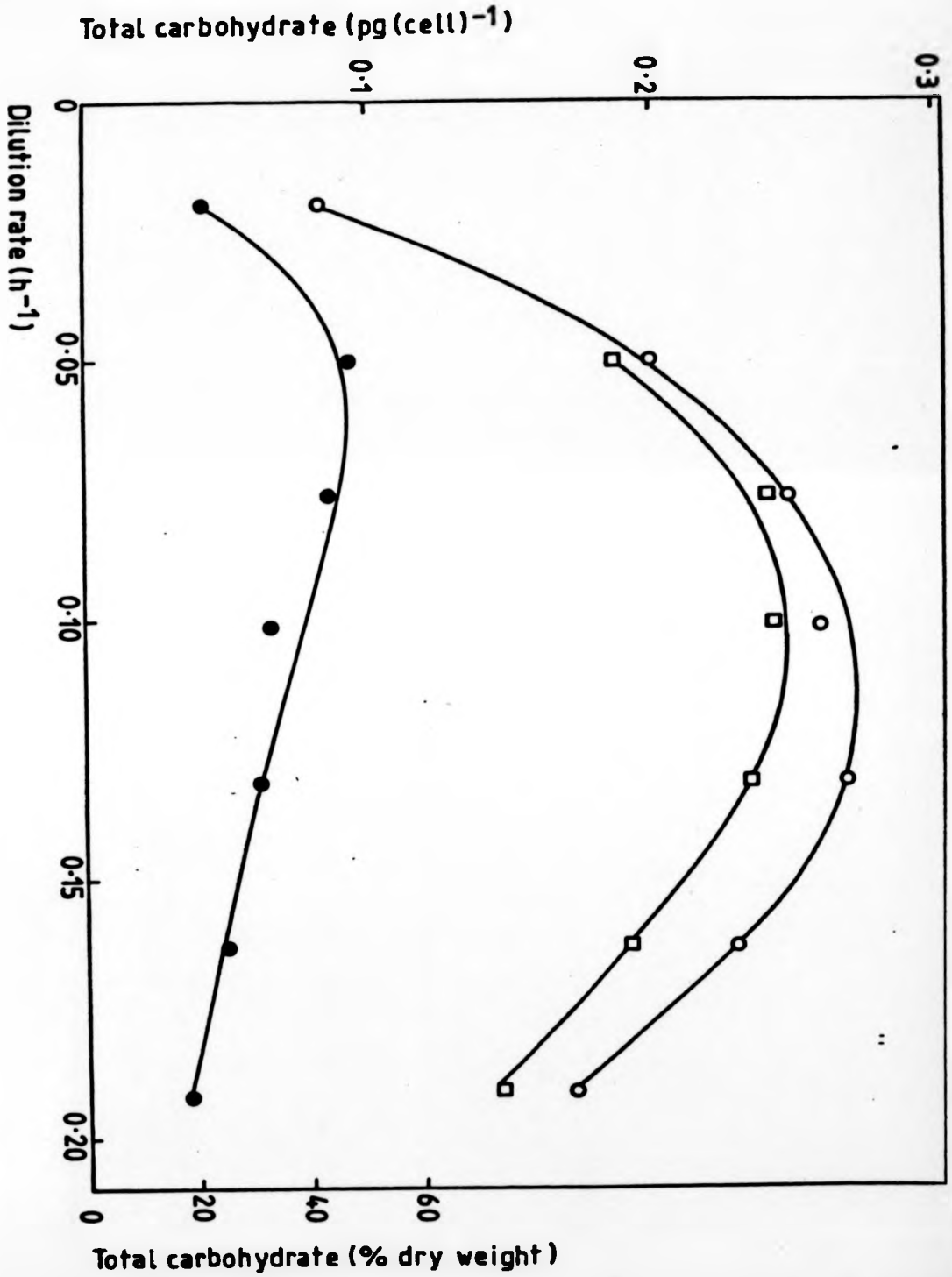
The carbohydrate content of A. nidulans, expressed as pg $(cell)^{-1}$ and as a percentage of the dry weight, was determined on a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The carbohydrate content per cell showed a continual increase with increasing growth rate although the actual values were very much lower than those obtained under light-limited conditions with a maximum value of only 0.083 pg carbohydrate $(cell)^{-1}$

Figure 4.16.

The influence of dilution rate on the total carbohydrate content, expressed as pg carbohydrate $(cell)^{-1}$ (○) and as a percentage of the dry weight (●), of A. nidulans grown in light-limited chemostat culture.

The results obtained using the anthrone method, expressed as pg carbohydrate $(cell)^{-1}$ (□), are also shown.


Total carbohydrate (pg (cell)$^{-1}$)
0·1
0·2
0·3
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0
20
40
60
Total carbohydrate (% dry weight)

at a dilution rate of 0.19 h^{-1} (figure 4.17). The carbohydrate content on a percentage dry weight basis showed a very slight increase with increasing growth rate from 8 - 14% (figure 4.17) with the values again being rather lower than those obtained under light-limited conditions.

4.5. LIPID COMPOSITION

4.5.1. Light-limited conditions

The lipid content of A. nidulans, expressed as pg $(cell)^{-1}$ and as a percentage of the dry weight, was determined on a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

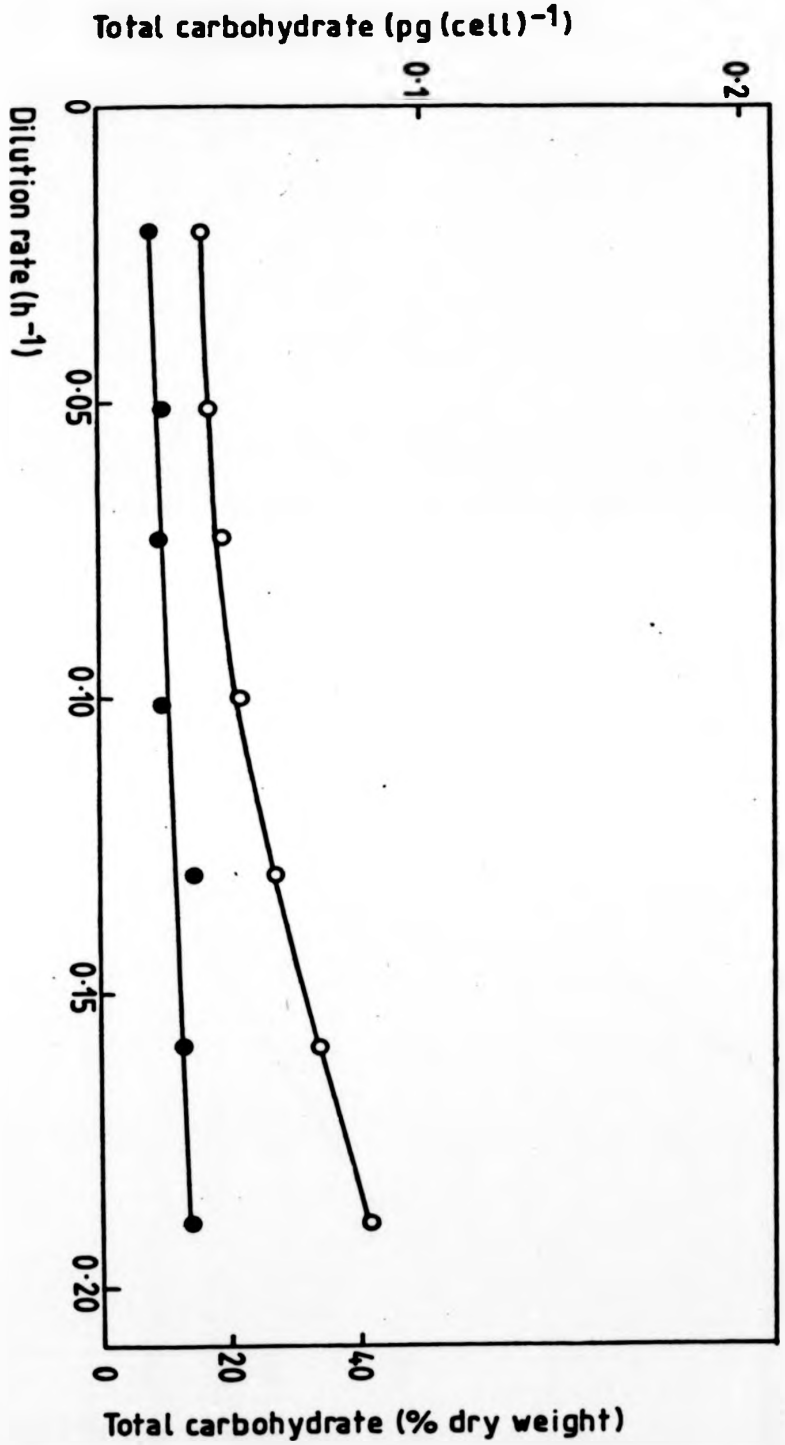
The lipid content per cell decreased to a minimum at a dilution rate of approximately 0.06 h^{-1} and then increased with increasing growth rate up to a maximum of 0.167 pg lipid $(cell)^{-1}$ at a dilution rate of 0.19 h^{-1} (figure 4.18). The lipid content on a percentage dry weight basis decreased to a minimum at a dilution rate of approximately 0.1 h^{-1} and then increased with increasing growth rate up to a maximum of 19% at a dilution rate of 0.19 h^{-1} (figure 4.18).

4.5.2. Carbon dioxide-limited conditions

The lipid content of A. nidulans, expressed as pg $(cell)^{-1}$ and as a percentage of the dry weight, was determined on a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

Figure 4.17.

The influence of dilution rate on the total carbohydrate content, expressed as pg carbohydrate $(cell)^{-1}$ (O) and as a percentage of the dry weight (●), of *A. nidulans* grown in carbon dioxide-limited chemostat culture.


Total carbohydrate (pg (cell)$^{-1}$)
0·1
0·2
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0
20
40
Total carbohydrate (% dry weight)

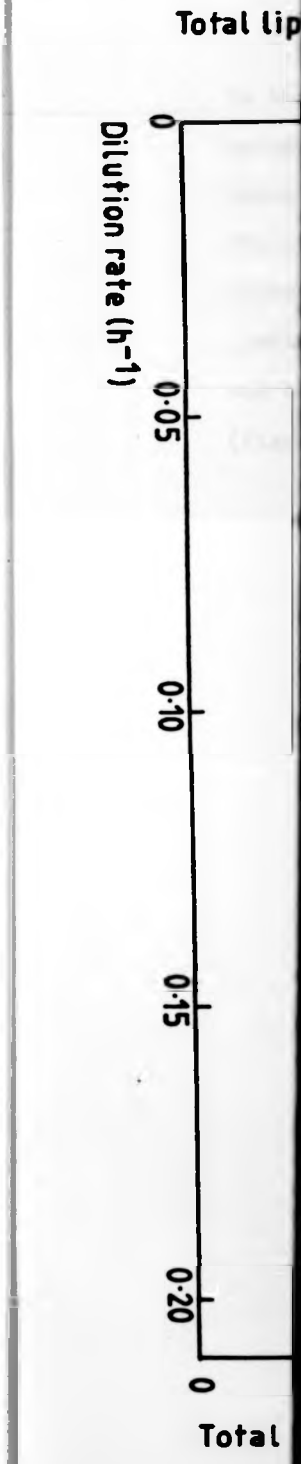


Figure 4.18.

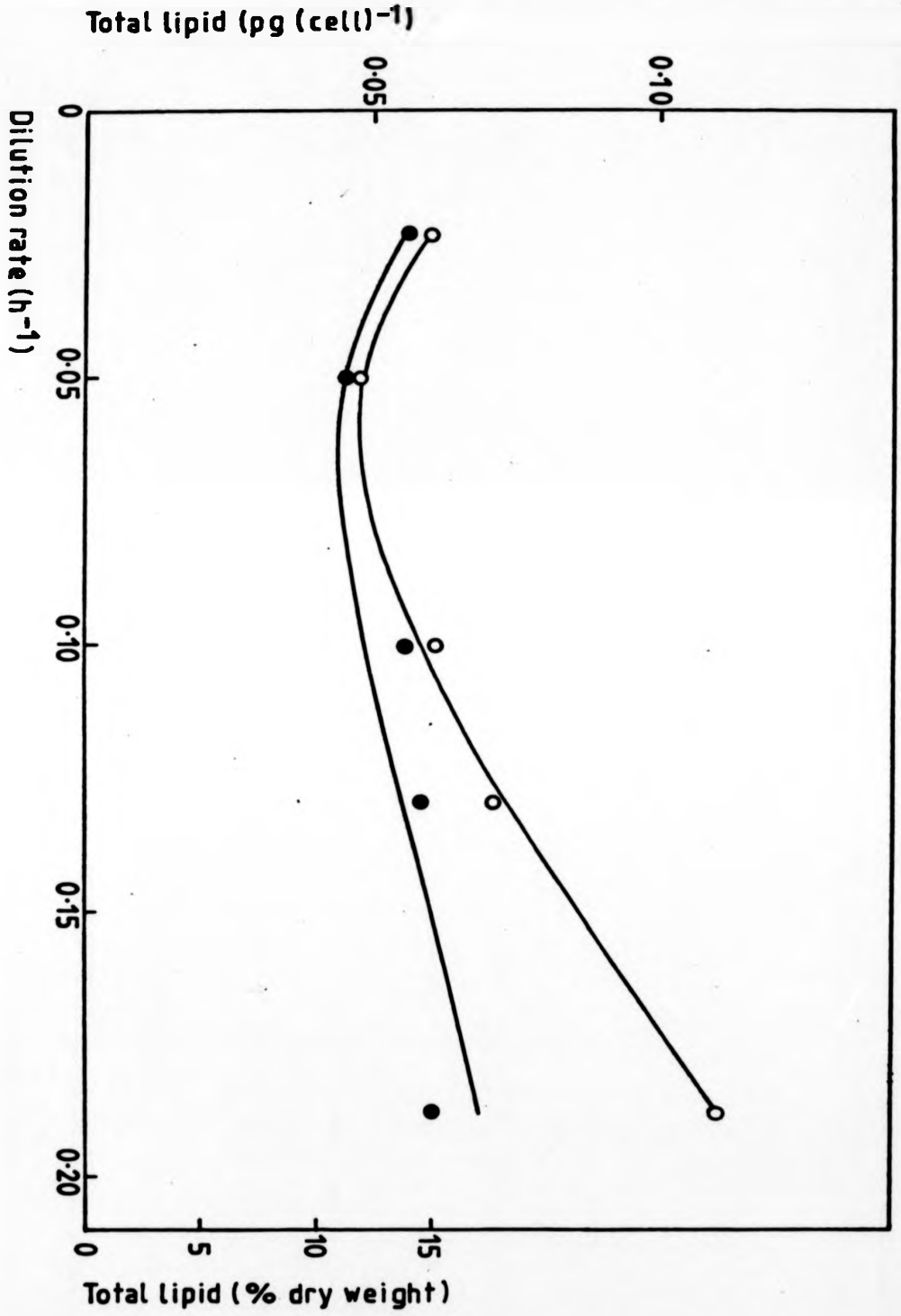
The influence of dilution rate on the total lipid content, expressed as pg lipid $(cell)^{-1}$ (O) and as a percentage of the dry weight (●), of A. nidulans grown in light-limited chemostat culture.

Total lipid (pg (cell)$^{-1}$)
0·05
0·10
0·15
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0
5
10
15
20
Total lipid (% dry weight)

The lipid content per cell showed a very similar pattern to that obtained under light-limited conditions although the actual values were lower, a maximum of 0.11 pg lipid $(cell)^{-1}$ being reached at a dilution rate of 0.19 h^{-1} (figure 4.19). The lipid content on a percentage dry weight basis again showed a very similar pattern to the obtained under light-limited conditions although in this case the minimum value was obtained at a dilution rate of approximately 0.06 h^{-1} (figure 4.19).

Figure 4.19.

The influence of dilution rate on the total lipid content, expressed as pg lipid $(cell)^{-1}$ (○) and as a percentage of the dry weight (●), of A. nidulans grown in carbon dioxide-limited chemostat culture.


Total lipid (pg (cell)$^{-1}$)
0·05
0·10
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0
5
10
15
Total lipid (% dry weight)

4.6. DISCUSSION

These results show that the amounts of all of the major macromolecular components of the cell change with differing environmental conditions. The components being shown to differ in their responses to changes in growth rate and the nature of the growth limiting factor. According to Herbert (1961) the chemical composition of a microorganism is directly and markedly affected by the environment and any environmental factor that affects growth will affect the macromolecular composition of the organism. Consequently, when giving a value for the chemical composition of a microorganism it is important to specify the environmental conditions precisely.

The results presented for the DNA, RNA and protein contents of A. nidulans have shown that growth rate in particular has a significant effect on the amounts of these macromolecules. The actual values, especially of RNA content, on a per cell basis under most conditions were comparable at similar growth rates under both light- and carbon dioxide-limitation. This is in agreement with the results stated by Herbert (1961) and Rosset, Julien and Monier (1966), for heterotrophic bacteria, who showed that the same pattern of changes seemed to occur for DNA, RNA and protein content whatever the nature of the limiting nutrient and concluded that the chemical composition of the cell was primarily dependent on the rate at which it was growing and was affected by the chemical composition of the growth medium

only in so far as this affected the growth rate. Similar results were also obtained by Rhee (1978), for the RNA content of the green alga Scenedesmus. who found that the RNA concentration reflected the growth rate irrespective of whether the limiting nutrient was nitrogen or phosphorus. However, different results were obtained when a physical factor, such as temperature, was altered. Schaechter, Maaløe and Kjeldgaard (1958) found that, although the growth rate of Salmonella typhimurium at 25°C was about half of that at 37°C, the mass,DNA and RNA remained nearly constant for a given medium and, therefore, concluded that the size and chemical composition of the cells were related to the growth rate only in so far as this depended on the growth medium.

The DNA, RNA and protein contents of A. nidulans in this study, with the exception of the patterns of DNA and protein changes on a per cell basis, showed qualitatively a similar response to their behaviour in heterotropic bacteria. Greater discrepancies were, in fact, shown from the previous study on this organism by Mann and Carr (1974). This was possibly due to the differing culture conditions used,since Mann and Carr (1974) employed batch culture techniques. In fact, in the present study different results were obtained for DNA and RNA content when batch rather than continuous culture growth conditions were used. Herbert (1961) also found slight differences in the patterns of response of the macromolecular content of the heterotrophic bacteria studied when they were grown under batch or continuous culture growth conditions. Karagouni (1979)

found that adequate periods of balanced growth during any stage of the growth cycle were difficult to achieve for A. nidulans grown in batch culture. Therefore, only transient, non-steady state conditions could be obtained making it difficult to correlate differences in macromolecular composition with the organism's growth rate. Furthermore, the influence of different nutrient limitations could not be examined satisfactorily in closed culture grown organisms as the growth medium composition was also changing throughout the course of the growth cycle. Therefore, it seems hardly surprising that the two different culturing conditions would tend to give different results for the cellular macromolecular content.

The decrease in DNA content of A. nidulans as a percentage of the total dry weight with increasing growth rate was in agreement with previous observations (Herbert, 1961) but in contrast to previous observations the DNA content per cell declined at growth rates above 0.1 h^{-1} (Herbert, 1961; Mann and Carr, 1974). Also, much lower values for the actual DNA content were obtained in the present study compared with the results of Mann and Carr (1974) who found that at mean generation times shorter than the C + D interval, where C is the time taken to replicate the chromosome and D is the time between the completion of replication and the subsequent cell division (Cooper and Helmstetter, 1968), the DNA content of the cell was already of the order of sixteen times greater than the DNA

content at zero growth rate. A C + D time of 178 min was obtained by determining the 'step up' time for cell division which was similar to the times of 65 and 115 min for C and D respectively for synchronous cultures of A. nidulans (Herdman, Faulkner and Carr, 1970) and similar to the values obtained by Karagouni (1979) from washout cultures. The data of Roberts, Klotz and Loeblich (1977) showed a similar trend suggesting that Agmenellum quadruplicatum contained multiple copies (2 or 3) of the genome as the basal DNA content, that is, for growth rates slower than the C + D time where a bacterial analogue would be expected to possess just one copy.

The genome size of A. nidulans has been determined to be 2.27×10^9 daltons (Herdman and Carr, 1974) and $2.12 \times 10^9 \pm 0.15$ daltons (Herdman, Janvier, Rippka and Stanier, 1979). A similar value of 2.08×10^9 daltons was obtained by extrapolation to zero growth rate of the results of Mann and Carr (1974). From these results a genome size of approximately 2.2×10^9 daltons can be assumed, indicating that the theoretical minimum cellular DNA content (that is, in a cell containing only one copy of the genome) should be 3.7 fg $(cell)^{-1}$. However, the minimum DNA content recorded in this study was 2.3 fg $(cell)^{-1}$ at the lowest dilution rate under light-limited conditions. This is obviously an impossible situation which was presumably due to experimental error. The main error was probably due to an overestimation of the cell numbers - the counting being

very subjective especially at low dilution rates when 'chains' of cells tended to occur (section 3.1.), also the DNA content may have been underestimated by the spectrophotometric method used. On the other hand, the above values for the genetic complexity for this organism could even be high.

If these values for genetic complexity are assumed to be approximately correct, the results from the present study indicate that at growth rates between 0.02 and 0.19 h^{-1} under both light- and carbon dioxide-limited continuous culture conditions there was no point at which more than two copies of the genome were present. This would be a more expected result than the results of Mann and Carr (1974) if the organisms were compared with the situation in heterotrophic bacteria (Donachie, Jones and Teather, 1973) as the shortest generation time of 3.65 hours (at μ=0.19 h^{-1}) was still greater than the C + D time. Consequently, a value of between one and two genomes would be expected. It seemed that the cells were not growing fast enough for multiple replication forks to occur as has been found in fast growing bacterial cells when a new 'round' of replication was initiated at the origin before the previous replication forks had reached the terminus (Helmstetter, 1968; Donachie *et al.*, 1973).

It has been reported that in slow growing cells, if the C + D time is not exceeded, DNA synthesis is discontinuous, the 'gap' periods being longer in the slowest growing cells (Pasternak, 1974) so that the DNA content of these cells would

be closer to the value for a non-replicating chromosome than to the value of one which is replicating or replicated (Lark, 1966). It was concluded by Lark (1966) that the time necessary for replicating DNA at very slow growth rates was regulated by both a reduction in the rate of DNA synthesis and an increase in the amount of time between chromosome replication cycles. This expected decrease in DNA synthesis at low growth rates was shown for A. nidulans under both light- and carbon dioxide-limitation when q_{DNA} was plotted against the growth rate (figure 4.3). The rate of DNA synthesis was found to increase with increasing growth rate as expected up to a maximum at a growth rate of approximately 0.1 h^{-1}. (The rate of increase being greater under carbon dioxide-limitation presumably because of the relatively smaller cell size under these conditions and the slightly higher DNA content.) Above a dilution rate of about 0.1 h^{-1} under both limitations the rate of DNA synthesis was constant with increasing growth rate indicating that the rate of synthesis was independent of the growth rate under these conditions. It seemed that the rate of synthesis of DNA could not be increased above this level as this was possibly being limited by the rate at which nucleotides could be added to the growing daughter strands at the replication forks - this being a finite value for bacteria (Donachie et al., 1973) and presumably so for this organism under these growth conditions. It could also be assumed that multiple replication forks did not occur

in contrast to the situation which seemed to occur under batch culture conditions for the same organism (Mann and Carr, 1974). As a consequence of the constant level of DNA synthesis above a dilution rate of 0.1 h^{-1} a decrease in the actual amount of DNA per cell was seen at fast growth rates (figures 4.1 and 4.5), multiple replication forks presumably not being needed as these rates were still less than the C + D time.

It is, however, difficult to determine accurately the rate of synthesis of a macromolecule simply by determining the changes in its amount since this is the net result of synthesis and degradation. A more direct method of determining the synthesis of DNA would have been to measure incorporation rates of radioactive precursors of this macromolecule, provided that the precursor was taken up and processed by the cells. The most common precursor used for DNA synthesis determinations is labelled thymidine which in most cases is incorporated exclusively into DNA (Glaser, Al-Nuri, Groshev and Shestakov, 1973). However, this precursor was found to be inefficiently assimilated by different strains of A. nidulans (Pigott and Carr, 1971; Glaser et al., 1973; Restaino and Frampton, 1975). Thymidine phosphorylase and thymidine kinase activities were even found to be absent in the same strain of A. nidulans as used in this study (Restaino and Frampton, 1975) indicating that the expected pathway of thymidine incorporation would not occur

in this organism. In contrast, using a different strain of A. nidulans (strain 1402-1) Ssymank, Kaushik and Lorenzen (1977) found that this organism readily incorporated [methyl-^{3}H] thymidine. However, due to the fact that precursors in general seemed to be inefficiently assimilated by A. nidulans or those that were more efficiently assimilated, such as radioactive uracil, were incorporated into both RNA and DNA (Pigott and Carr, 1971; Glaser et al., 1973) it was decided that it would be difficult to interpret quantitatively any results obtained so this method of determining the rate of DNA synthesis was not attempted. It seems possible that the relatively poor assimilation rates of DNA precursors has been a major factor in the lack of information available concerning the mechanism and regulation of DNA synthesis in cyanobacteria.

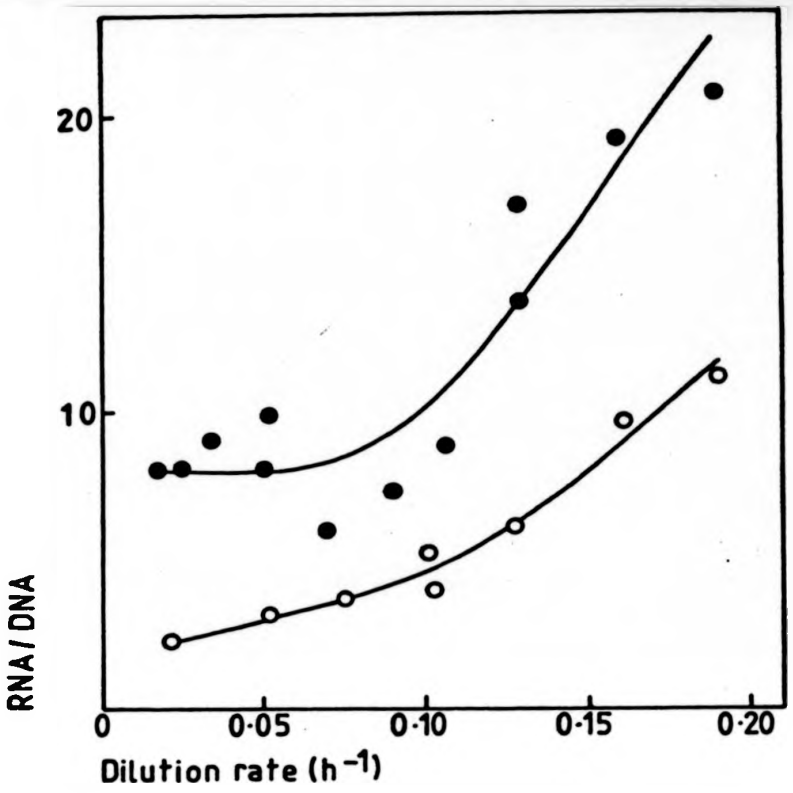
The behaviour of the RNA component with changing growth rate was similar to that obtained by Mann and Carr (1974) although an exponential increase was not seen. The results on a per cell and percentage dry weight basis were also similar to results obtained for heterotrophic bacteria. The greatly increased levels of RNA relative to DNA shown at fast growth rates when the RNA/DNA ratio was plotted (figure 4.20) indicated non-coordinate synthesis of these two components and was in agreement with results obtained for heterotrophic bacteria. The increased synthesis of RNA at fast growth rates relative to DNA was also indicated by the linear increase in RNA synthesis

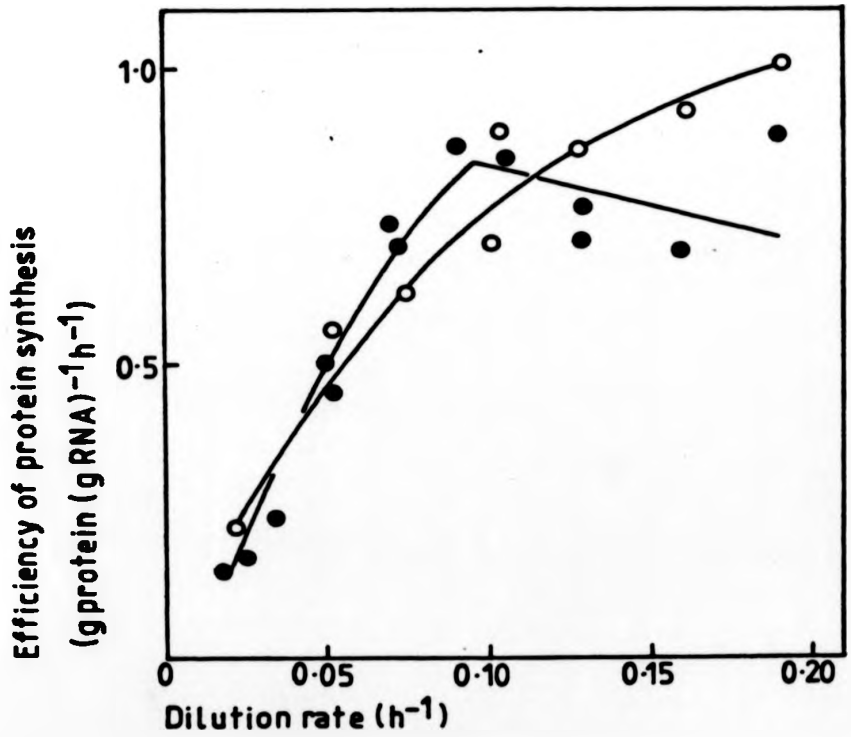
Figure 4.20.

The influence of dilution rate on the RNA/DNA ratio for *A. nidulans* grown in light-limited (●) and carbon dioxide-limited (○) chemostat culture.

Figure 4.21.

The influence of dilution rate on the efficiency of protein synthesis (g protein (g RNA)$^{-1}$ h^{-1}) for *A. nidulans* grown in light-limited (●) and carbon dioxide-limited (○) chemostat culture.


20
10
RNA / DNA
0
0·05
0·10
0·15
0·20
Dilution rate (h⁻¹)


1·0
0·5
Efficiency of protein synthesis
(g protein (g RNA)⁻¹h⁻¹)
0
0·05
0·10
0·15
0·20
Dilution rate (h⁻¹)

with increasing growth rate compared with the levelling off of DNA synthesis at growth rates faster than 0.1 h^{-1}. The results of work on heterotrophic bacteria indicated that these increased levels of RNA were due to an increase in the relative level of ribosomal RNA (rRNA) with increasing growth rate (Kjeldgaard and Kurland, 1963; Maaløe and Kjeldgaard, 1966; Rosset et al., 1966; Kjeldgaard, 1967; Gray and Midgley, 1970; Skjold, Juarez and Hedgcoth, 1973), indicating that transcriptional control was being shown due to alterations in the relative rates of synthesis of the different RNA species in cultures with different generation times. In contrast to these results, Mann and Carr (1974) showed that the RNA/DNA ratio for A. nidulans was independent of the growth rate and that the relative percentages of rRNA and transfer RNA (tRNA) did not alter with changes in growth rate (Mann and Carr, 1973). These authors concluded that A. nidulans was unable to respond to altered growth rates by alteration in the relative frequency of transcription of the stable RNA genes which was consistent with previous findings of an apparent lack of selective gene expression in this organism (Carr, 1973a). However, the results of the present study seemed to indicate similar control at the level of transcription for A. nidulans as shown by heterotrophic bacteria, but obviously to be sure of this the relative amounts of rRNA and tRNA would have to be determined for this organism under the growth conditions used.

Another indication that A. nidulans might be subject to

transcriptional control was the accumulation of two unusual guanosine nucleotides, guanosine 5'-diphosphate-3'-diphosphate (ppGpp) and guanosine 5'-triphosphate-3'-diphosphate (pppGpp) with a reduction in the rate of stable RNA accumulation (Mann, Carr and Midgley, 1975; Smith, 1977; Smith and Carr, 1977). ppGpp and pppGpp accumulation were implicated in the control of the accumulation of stable RNA species and the regulatory mechanism seemed to be elicited by reduced aminoacylation of tRNA and appeared to act at the level of stable RNA chain initiation. Smith (1979) found that the concentration of ppGpp increased exponentially with decreasing growth rate in A. nidulans grown at different light intensities. It would be expected that an inhibitory regulator would vary inversely with growth rate and RNA content. However, this is inconsistent with the concept of ppGpp as a regulator of transcription in A. nidulans according to the results of Mann and Carr (1974) who showed a constant ratio of RNA to DNA with increasing growth rate, implying a constant rate of transcription from individual cistrons. Therefore, the rate of initiation of RNA polymerase on promoter sites at stable RNA cistrons would be expected to remain constant irrespective of the growth rate and no modulation of this process would be required. Smith (1979) concluded that the significance of the relationship between the ppGpp concentration and RNA content was reduced to the level of coincidence and detracted from the concept of ppGpp as a regulator of stable RNA accumulation. However, the results

obtained in the present study for A. nidulans show a higher RNA/DNA ratio at faster growth rates suggesting an increased rate of transcription per stable RNA cistron so this would be consistent with the concept of ppGpp as a regulator of transcription.

The results from the transition experiments substantiated the view that A. nidulans had more control over transcription and translation than previously thought (Mann and Carr, 1974). It was indicated by these experiments that this control was real, being exerted by a single population, rather than the possibility of two different sub-types. Although the actual values obtained for DNA and RNA on a per cell basis differed from the results expected for the corresponding steady state growth rates that the organisms were shifted to (probably due to the transient state conditions), when the rates of synthesis of these macromolecules, in terms of q_{DNA} and q_{RNA}, were determined (figures 4.3. and 4.4. respectively) the values obtained, especially for the rate of RNA synthesis, were very close to those expected for the growth rates concerned. This rapid adaptation to a new growth rate would not be expected if, in fact,two different sub-types were present as the original population would still be expected to be dominant after this short period of time assuming that the other population could not adapt quickly enough to the new growth rate to resume dominance. These results were essentially in agreement with shift experiments carried out on heterotrophic bacteria which showed that RNA reacted

most readily to sudden changes in growth conditions (Kjeldgaard, Maaløe and Schaechter, 1958; Neidhardt and Magasanik, 1960; Kjeldgaard, 1961; Maaløe and Kjeldgaard, 1966). It was shown that a shift-up to a more complex medium resulted in an immediate increase in RNA synthesis, generally to a value above that characteristic of the new medium, but the preshift rate of DNA synthesis was maintained throughout the round of replication in progress at the time, the cells dividing at the end of this round and the next round of synthesis proceeding at the new rate. Similarly, a shift-down to a poorer medium was characterised by an immediate cessation of RNA synthesis with the preshift rates of cell division and DNA synthesis being maintained. However, these immediate effects were followed by slow and gradual rate changes that eventually led to balanced growth at a reduced rate characteristic of the new medium.

The patterns of change of protein content of A. nidulans shown with growth rate were essentially similar to those obtained by Karagouni (1979) for the same organism grown under the same conditions of light-and carbon dioxide-limitation. Also, the general trend towards a decrease in protein content on a percentage dry weight basis with increasing growth rate was comparable with the protein content changes in heterotrophic bacteria as was the fact that the DNA and protein content curves followed a similar pattern with changes in growth rate

(Herbert, 1961).

At high growth rates the relative level of RNA in this study was shown to be greatly increased relative to the cellular protein levels, a phenomenon that has also been noted with respect to heterotrophic bacteria. The proportion of RNA as tRNA was found to decrease with increasing growth rate (Kjeldgaard and Kurland, 1963; Maaløe and Kjeldgaard, 1966; Rosset et al., 1966; Kjeldgaard, 1967; Gray and Midgley, 1970; Skjold et al., 1973) and it was concluded that the limited availability of tRNA, when compared with the number of ribosomes available could be limiting protein synthesis in the cell. The data supports the idea that a portion of the ribosomes are idle indicating that only a minor fraction would at any given time be actively engaged in the process of polypeptide synthesis. Maaløe and Kjeldgaard (1966) therefore suggested that the ribosomes may go through a phase of activation as a prerequisite for specific combination with the mRNA strand to initiate the polypeptide assembly process. The data, as well as the work of Norris and Koch (1972), also implicated non-coordinate control of the stable RNA species which was in contrast to the results of Mann and Carr (1973) for A. nidulans who found that the relative percentages of rRNA and tRNA did not alter with changes in growth rate. This result being consistent with the fact that a constant DNA to RNA ratio was shown for this organism (Mann and Carr, 1974). However in the present study

the results seemed to be more comparable to those obtained for heterotrophic bacteria so it would perhaps be expected that the tRNA and rRNA levels might alter with changes in growth rate under these growth conditions if, in fact, these determinations were carried out.

The efficiency of protein synthesis, calculated as the rate of cellular protein synthesis per unit mass of RNA (that is, (protein/RNA) x μ) at each growth rate, was determined for several heterotrophic bacteria with differing results being obtained. Skjold et al. (1973) found that up to a generation time of 1.5 generations per hour the efficiency for both tRNA and rRNA for Escherichia coli increased linearly with increasing growth rate but beyond that rate the efficiencies decreased. On the other hand, Rosset et al. (1966) found that the efficiencies increased regularly with growth rate for E. coli and S. typhimurium whereas Kjeldgaard and Kurland (1963) found that efficiencies for S. typhimurium remained essentially constant at all growth rates for rRNA but efficiencies increased with increasing growth rate for tRNA.

The efficiency of protein synthesis for total RNA was plotted from the results of this study for A. nidulans (figure 4.21). For light-limited organisms the efficiency increased up to a growth rate of approximately 0.1 h^{-1} and then showed a slight decrease with further increase in growth

rate - these results being comparable to those of Skjold et al. (1973). On the other hand, the efficiency showed a continual increase with increasing growth rate for carbon dioxide-limited organisms - this being comparable to the results of Rosset et al. (1966). This continual increase was presumably due to the increasing amount of carbon being added to the growth medium which could be used in protein formation and which did not reach a saturating concentration even at the highest growth rates. These results seemed to indicate that the rate of protein synthesis was very dependent on the composition of the growth medium, especially with regard to the limiting substrate.

The results presented for the pigment composition of A. nidulans, in agreement with the nucleic acid and protein results, also showed that growth rate in particular had a significant effect on the amounts of these macromolecules. The actual values, on a per cell basis, were, in general, comparable at similar growth rates under both light-and carbon dioxide-limitation. The dry weight values obtained under carbon dioxide-limiting conditions being again slightly higher than under light-limitation due to the relatively smaller cell size under these growth conditions.

The results showed that the phycobiliprotein phycocyanin was the major contributor to the total pigment content of this organism (A. nidulans being known to contain no phycoerythrin (Chapman, 1973)) with total carotenoids making only a

minor contribution. It was also shown, using the equations of Arnon (1949), that this organism contained no chlorophyll b. This was in agreement with previous reports (Emerson and Lewis, 1942; Myers and Kratz, 1955; Fogg, Stewart, Fay and Walsby, 1973; Stanier and Cohen-Bazire, 1977) that chlorophyll b was not present in cyanobacteria.

Under both limitations on a per cell basis it was shown that the phycocyanin and chlorophyll contents of A. nidulans increased rapidly at low dilution rates but then less rapidly as the growth rate was further increased and, in most cases, even decreased at the highest growth rates. This increase would be expected as the cells were increasing in size and the numbers of cells per ml were decreasing (sections 3.3. and 3.4.) so there was more light available per cell (the light intensity remaining constant at all growth rates). However, at the fastest growth rates there was presumably a greater possibility of capturing the available light so perhaps the actual amounts of pigment per cell did not need to be so high or, on the other hand, the amounts of pigments might have decreased so as to balance the light and dark reactions of photosynthesis. A decrease in carbon dioxide fixation was, in fact, shown at high growth rates of this organism (Karagouni, 1979; Karagouni and Slater, 1979).

On a dry weight basis under light-limitation, there was a general decrease in the amounts of phycocyanin and chlorophyll with increasing growth rate, except at the lowest dilution

rates. This was in agreement with the results of Myers and Kratz (1955) and although these workers found higher actual percentage values of these pigments for A. nidulans (which might be expected due to differing culture conditions) the ratio values obtained were very similar, ranging from 9.3 to 7.7, showing little variation with changing growth rate. At the lowest dilution rates under light-limited conditions a second nutrient became limiting (section 3.3.) which was possibly nitrate. If this was so, then low levels of the nitrogen containing pigments would, in fact, be expected (Allen and Smith, 1969; van Gorkom and Donze, 1971; DeVasconcelos and Fay, 1974; Lau, MacKenzie and Doolittle, 1977). Despite the low actual levels of pigments at these low growth rates, the phycocyanin/chlorophyll ratio was still relatively constant indicating that the organisms strive to maintain this value as presumably photosynthesis is most efficient under these circumstances with the majority of the light absorbed by phycocyanin being utilised and the two photosystems being in balance.

Under carbon dioxide-limited conditions, except at very low growth rates, the phycocyanin/chlorophyll ratios were almost the same at corresponding growth rates to those obtained under light-limited conditions. This again indicates that this is the most efficient condition for photosynthesis. At very low growth rates, below 0.07 h^{-1}, under carbon dioxide-limitation there was a pronounced decrease in the amount of

phycocyanin present which led to a much lowered phycocyanin/chlorophyll ratio. This was presumably due to the fact that carbon dioxide starvation was most pronounced at these low dilution rates and is in agreement with the work of Eley (1971) who showed a decrease in the phycocyanin/chlorophyll ratio with a decrease in carbon dioxide concentration and Goedheer (1976) who stated that insufficient carbon dioxide supply to cope with growth at high irradiances appeared to inhibit phycocyanin formation.

Under both limitations the total carotenoids/chlorophyll ratios obtained were low and very similar under most growth conditions. Similar values of this ratio were obtained for A. nidulans by Myers and Kratz (1955) and Eley (1971) of between 0.4 and 0.6 and for A. quadruplicatum of 0.56 (Parsons, Stephens and Strickland, 1961). However, the total carotenoid content of A. nidulans did not tend to follow the patterns shown by the other two pigments with changing growth rate and, in fact, seemed to increase when the phycocyanin content decreased as also shown by Halldal (1958). This phenomenon was most pronounced under carbon dioxide-limited conditions at low growth rates with the decrease in phycocyanin content which corresponded to a two-fold increase in the carotenoid/chlorophyll ratio. It was possible that the increase in carotenoids compensated for the loss of phycocyanin and that under these conditions the carotenoids were taking the

place of phycocyanin as accessory pigments. It seems that carotenoids are not as efficient as phycocyanin as accessory pigments in cyanobacteria but could possibly act in a photosynthetic role under certain conditions (Emerson and Lewis, 1942). On the other hand, the increase in carotenoid content could be attributed to an increased protective function of these pigments of the photosynthetic apparatus (Halldal, 1958; Nakayama, 1962) due to the decrease in phycocyanin content. In this case the light reactions would presumably be reduced so as to match the dark reactions (which would be lowered due to the limited CO_2 availability) as suggested by Eley (1971).

These results showed that under most conditions of growth, irrespective of the growth rate or limiting substrate, only small variations occurred in the ratios of the pigments chlorophyll a/phycocyanin/total carotenoids in A. nidulans so substantiating the view of Jones and Myers (1965) and Myers, Graham and Wang (1978) that large variations in these ratios only occur under extremely adverse conditions, such as shown in these experiments by pronounced carbon dioxide starvation. Under most growth conditions the organism seemed to maintain relatively constant pigment ratios which were presumably the most efficient for photosynthesis and subsequent growth.

The results presented for the carbohydrate composition of A. nidulans showed that substrate-limitation as well as growth rate had a significant effect on the amounts of this macromolecule. This, and the fact that the carbohydrate

composition reached very high percentage values, up to a maximum of 47%, under light-limited conditions, indicated that this was the main storage product in this organism. The very low values of carbohydrate shown under carbon dioxide-limited conditions also indicated the storage function of this compound, as under extreme carbon limitation the storage polysaccharide would not be expected to accumulate - the available carbon being used primarily for growth and cell division rather than for maintenance functions in agreement with the results of Cook (1963). The values did tend to increase slightly with increasing growth rate, much of this probably being due to the ribose content of RNA which was shown to increase with increasing growth rate on both a per cell and dry weight basis (section 4.1.2. and figure 4.6). However, the values on a per cell basis even at the highest growth rates were still under a half of those obtained under light-limited conditions. This indicates that the cells could survive even if they contained only very small amounts of a storage material, such as carbohydrate, in contrast to the basal materials, such as nucleic acids and proteins, which are essential and must be present in near optimum amounts under prevailing environmental conditions. This is in agreement with the conclusions of Herbert (1961).

The pattern of carbohydrate accumulation on a dry weight basis under light-limitation was very similar to that shown by Aiking and Sojka (1979) for Rhodopseudomonas capsulata.

There did not seem to be an excessive accumulation of carbohydrate at the lowest dilution rates, that is, below 0.05 h^{-1}, where if nitrogen was the second limiting factor (section 3.3) this would be expected. However, an increase was shown in the carbohydrate content as growth rate increased up to a dilution rate of just above 0.06 h^{-1} which was followed by a rapid decrease on a dry weight basis. A similar decrease in carbohydrate content was also shown on a per cell basis indicating either a more rapid turnover of carbohydrate or a reduced rate of synthesis. This might be expected at high growth rates when presumably more of the available carbon and energy was needed for rapid growth and cell division rather than for maintenance and storage functions.

The results presented for the lipid composition of A. nidulans show that, like the nucleic acid, protein and pigment results, growth rate in particular had a significant effect on the amounts of this macromolecule. Similar results were obtained under both limitations although the actual values on a per cell basis under carbon dioxide-limitation were slightly lower in agreement with the reduced lipid content shown for Synechococcus lividus due to carbon dioxide deprivation (Miller and Holt, 1977). The similar patterns under both limitations and the fact that the percentage values of lipid were lower than those for carbohydrate indicated that these compounds did not have a major storage function in this organism. However, at the very lowest dilution

rates under both limitations the lipid values were perhaps slightly higher than expected, so this perhaps indicated a storage function at these very low growth rates. This would perhaps be expected under light-limited conditions if nitrogen was limiting at these rates (section 3.3) especially as little accumulation of the other possible nitrogen-deficient compound, carbohydrate, occurred. This would, however, be unusual as it seems that cyanobacteria, in contrast to many other micro-organisms, do not tend to accumulate lipid material under nitrogen-limiting conditions (Collyer and Fogg, 1955; van Baalen and Marler, 1963). It is not known why lipid should accumulate slightly at low growth rates under carbon dioxide-limiting conditions as it would be expected that the very low carbon levels at these rates would be used for the production of more essential components, such as nucleic acids and proteins.

The lipid component in general increased with increasing growth rate which would be expected as lipid is a major component of the membranes (cytoplasmic and thylakoidal) and the organisms were shown to increase in size with increasing growth rate (sections 3.3. and 3.4.). The fact that on a per cell basis the amounts of chlorophyll a and carotenoids (section 4.3.) increased with increasing growth rate also indicated an increase in the thylakoid membrane with increasing growth rate. However, at the highest growth rates the chlorophyll and carotenoids tended to level off or even

decrease slightly so possibly leading to a slight decrease in the amount of thylakoid membrane (section 5) at these growth rates but this may have been compensated for by the rapid increase in cell size, especially under light-limited growth, leading to an increased production of cytoplasmic membrane. On the other hand, at these high growth rates, the storage function of the lipid component may have increased due to the lowered amounts of carbohydrate present.

The sum of the percentages of the protein, carbohydrate and lipid constituents, plus the ash content, should give a recovery of 100%. In this study the ash content of A. nidulans was not determined but if an average value of 15% obtained by Collyer and Fogg (1955) or the value of 10.7% for A. quadruplicatum obtained by Parsons et al. (1961) is assumed then a recovery of almost 100% was obtained at most growth rates under both limitations (tables 4.3 and 4.4). However, very low values of 62 and 64% recovery were obtained respectively at dilution rates of 0.19 h^{-1} under light-limited conditions and 0.02 h^{-1} under carbon dioxide-limited conditions. Similar deviations from a recovery of 100% were obtained by Collyer and Fogg (1955) and Parsons et al. (1961) which in general could be explained by the use of average constants for the conversion of biological values which would not, perhaps, be applicable in every case, the presence of various undetermined inorganic constituents and other unestimated material, probably cell wall material, which would not be hydrolysed under the conditions used or

TABLE 4.3. Total macromolecular composition of A. nidulans grown in light-limited chemostat culture

Dilution rate (h^{-1})	Protein (% dry weight)	Carbohydrate (% dry weight)	Lipid (% dry weight)	Total % composition
0.02	41.32	20.92	15.42	77.66
0.05	40.41	47.32	12.22	99.95
0.10	44.00	32.69	8.99	85.68
0.13	36.63	31.00	12.00	79.63
0.19	25.62	17.75	18.79	62.16

TABLE 4.4. Total macromolecular composition of A. nidulans grown in carbon dioxide-limited chemostat culture

Dilution rate (h^{-1})	Protein (% dry weight)	Carbohydrate (% dry weight)	Lipid (% dry weight)	Total % composition
0.02	41.44	8.39	14.00	63.83
0.05	56.09	10.05	11.78	77.92
0.10	54.48	10.08	15.80	80.36
0.13	53.73	15.80	14.52	84.05
0.19	45.63	13.75	15.09	74.47

which did not yield reducing products on hydrolysis. In the present study much of the deviation could probably be attributed to the generality of the assay methods used which would perhaps give different results at different growth rates under different growth conditions. For example, the protein estimation method used (Lowry, Rosebrough, Farr and Randall, 1951) measured the presence of tryptophan residues - so the actual proteins present may not have contained an average number of these residues or may have changed composition at different growth rates. A similar problem may have occurred by using the phenol reagent as a 'universal carbohydrate method' as it is virtually impossible to determine all types of carbohydrate (including free sugars, simple and complex polysaccharides and complex macromolecules) with a single reagent (Herbert et al., 1971). However, the lipid determinations probably accounted for the greatest quantitative error as the dry weight rather than a colourimetric method of measurement was used. Nevertheless, despite possible errors, most of the cell dry weight did seem to be accounted for so the errors could not have been excessive..

PART 5. ULTRASTRUCTURE OF ANACYSTIS NIDULANS GROWN UNDER CHEMOSTAT CONTINUOUS-FLOW CULTURE CONDITIONS

5.1. FINE STRUCTURE OF A. NIDULANS

The cells of A. nidulans, as examined under the electron microscope, showed a typical prokaryotic type of cellular organisation (plate 5.1; section 1.3.1.).

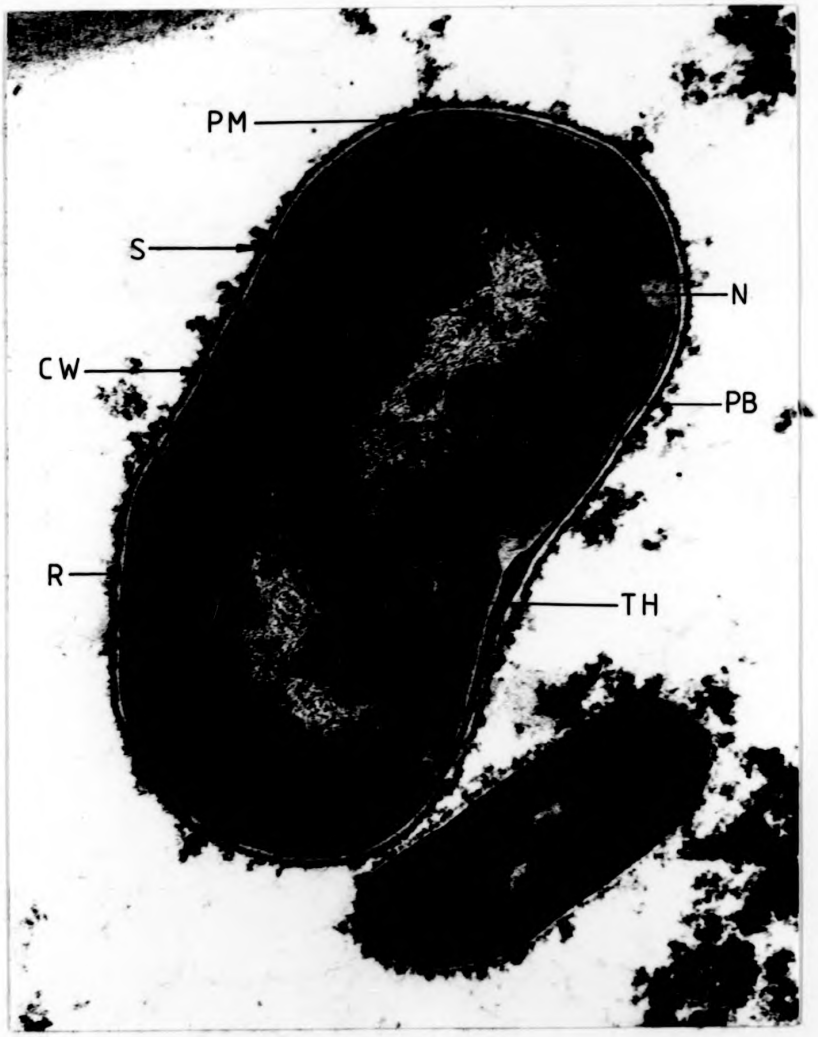
An irregular electron dense overlayer was seen on the outside of the cell envelope similar to that found for A. nidulans by Allen (1968b) who presumed that this was a thin sheath. Inside this was the cell wall which appeared as a multilayered structure similar to that found in the same organism by Allen (1968b) and Golecki (1977). The plasma membrane was found to surround the cytoplasm which contained generally three or four peripheral thylakoids orientated parallel to the longitudinal cell wall. This was in agreement with the work of Ris and Singh (1961), Echlin (1964b) and Allen (1968a) for A. nidulans. The thylakoids showed the typical tripartite structure with the membranes in the flattened vesicles being closely apposed to each other and not separated by an intrathylakoidal space. Various granular inclusions were seen amongst the thylakoids although it was impossible to distinguish between them due to the unspecificity of the staining procedure used.

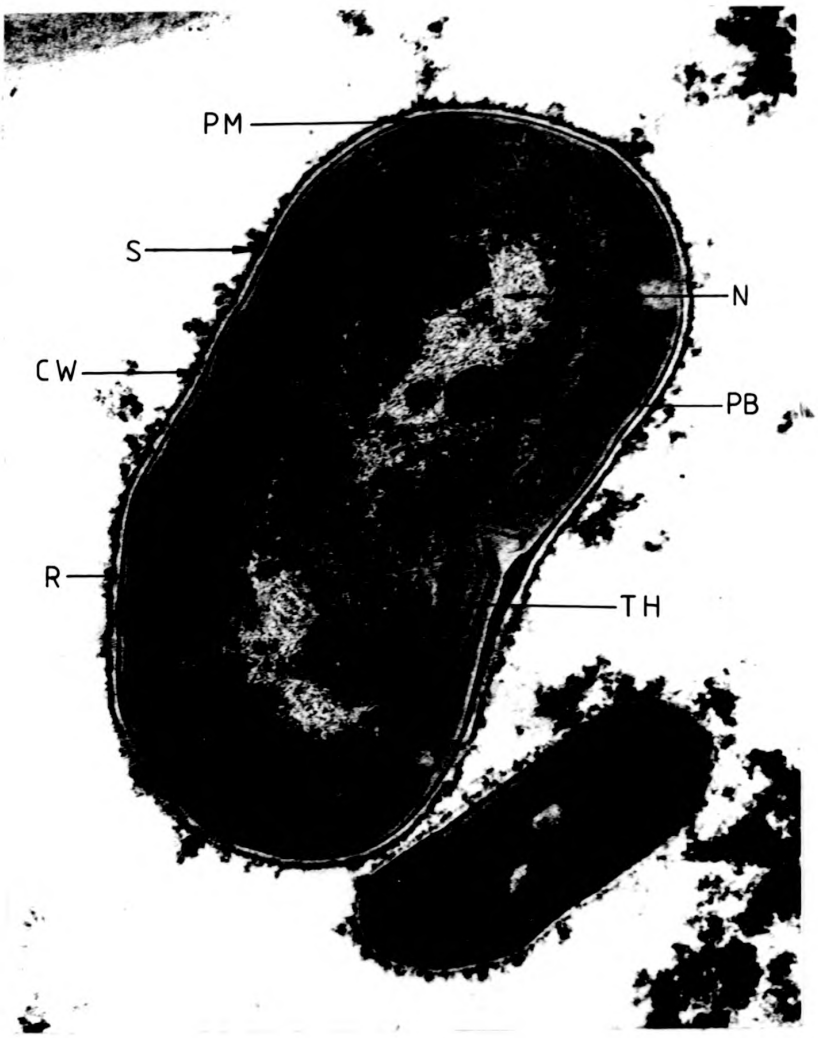
The nuclear material was found to occupy the central region of the cell giving an almost homogeneous fine-fibrillar appearance similar to that found by Leak (1967), Fogg, Stewart, Fay and Walsby (1973) and Fuhs (1973) when the Kellenberger method of fixation (Kellenberger, Ryter and Sechaud, 1958) was used.

Plate 5.1.

The ultrastructure of *A. nidulans* grown at D=0.10 h^{-1} in light-limited chemostat culture (X 72500).

S, sheath; CW, cell wall; PM, plasma membrane; TH, thylakoids; N, nuclear region; R, ribosomes; PB, polyhedral body.


PM
S
N
CW
PB
R
TH


PM
S
N
CW
PB
R
TH

Ribosomes were found to be especially concentrated around the nucleoplasmic region, in close contact with the DNA fibrils, in agreement with the results of other workers (Ris and Singh, 1961; Lang, 1968; Fogg et al., 1973; Fuhs, 1973).

The large polygonal bodies located in the nuclear region were probably polyhedral bodies (carboxysomes) due to their shape, size and position within the cell. This being in agreement with the fact that, according to Shively (1974), these bodies seem to be present in all organisms that can utilise carbon dioxide as the sole carbon source and that fix carbon dioxide via the reductive pentose phosphate pathway.

The cell shown in plate 5.1. was in the process of dividing. According to Allen and Stanier (1968) rod shaped unicellular organisms such as A. nidulans multiply by regular binary transverse fission in a single plane perpendicular to the long axis of the cell. In this case a unilateral invagination of the photosynthetic lamellae was seen. This was in agreement with the results of Allen (1968b) who found that this process was the first sign of cell division in this organism and that symmetrical invagination of the cytoplasmic membrane and the two inner wall layers followed. The outer wall layers were found not to be involved in septum formation. The septum was therefore formed by centripetal growth as is characteristic of bacteria but not of eukaryotic algae in which centrifugal septum formation occurs. In unicellular cyanobacteria, such as A. nidulans, the daughter cells were found to become isolated

from one another by constriction of the outer wall layers and by gradual splitting of the newly formed septum (Fogg et al., 1973).

5.2. LIGHT-LIMITED CONDITIONS

The ultrastructure of A. nidulans was examined in steady state cultures at dilution rates of 0.02, 0.10 and 0.19 h^{-1}.

The fine structure of the cells was basically the same irrespective of the dilution rate although some of the cellular components did alter quantitatively. At a dilution rate of 0.02 h^{-1} the cells contained in general three to four peripheral thylakoids and generally one but sometimes two polyhedral bodies (plate 5.2.). At a dilution rate of 0.10 h^{-1} the cells contained in general three to five peripheral thylakoids and mainly two but sometimes up to four polyhedral bodies (plates 5.1. and 5.3.). At a dilution rate of 0.19 h^{-1} the cells contained possibly slightly fewer peripheral thylakoids normally three to four per cell and generally one or two polyhedral bodies (plate 5.4.). Also at this dilution rate the nucleoplasmic region of the cells was consistently more granular in appearance indicating an increased number of ribosomes.

5.3. CARBON DIOXIDE-LIMITED CONDITIONS

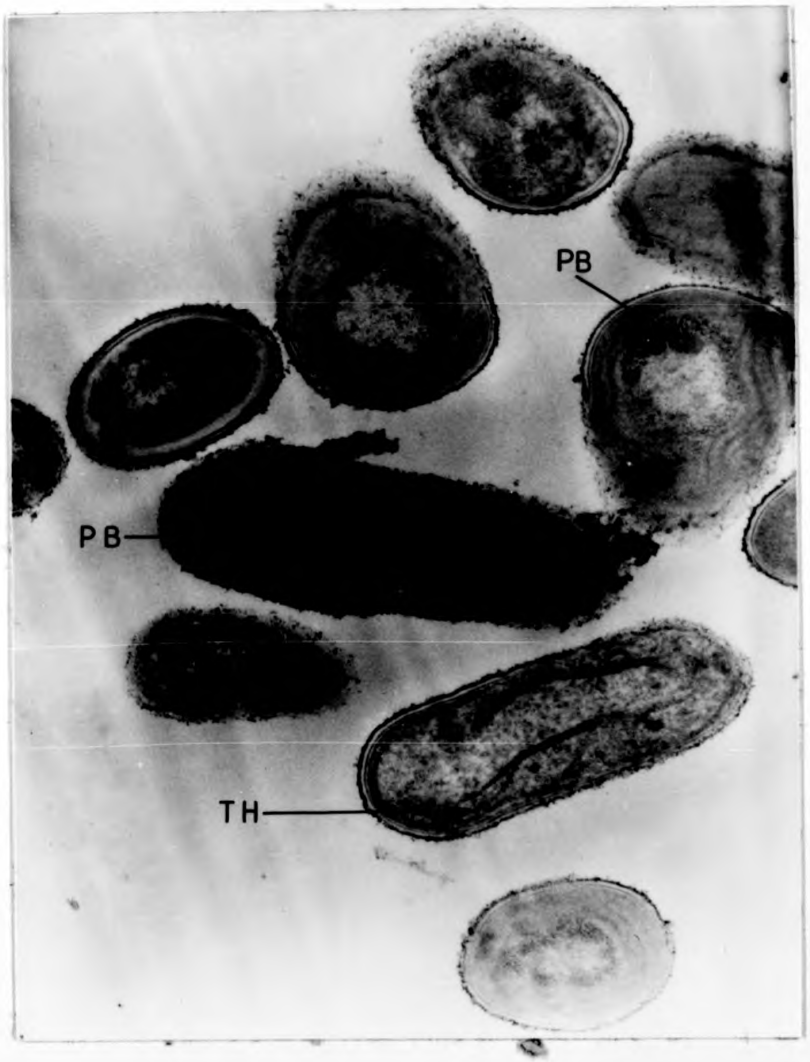
The ultrastructure of A. nidulans was examined in steady state cultures at dilution rates of 0.02, 0.10 and 0.19 h^{-1}.

The fine structure of the cells was basically the same as those grown under light-limited conditions except for the presence

Plate 5.2.

The ultrastructure of *A. nidulans* grown at D=0.02 h^{-1} in light-limited chemostat culture (X 37,500).

TH, thylakoids; PB, polyhedral body.


PB
PB
TH

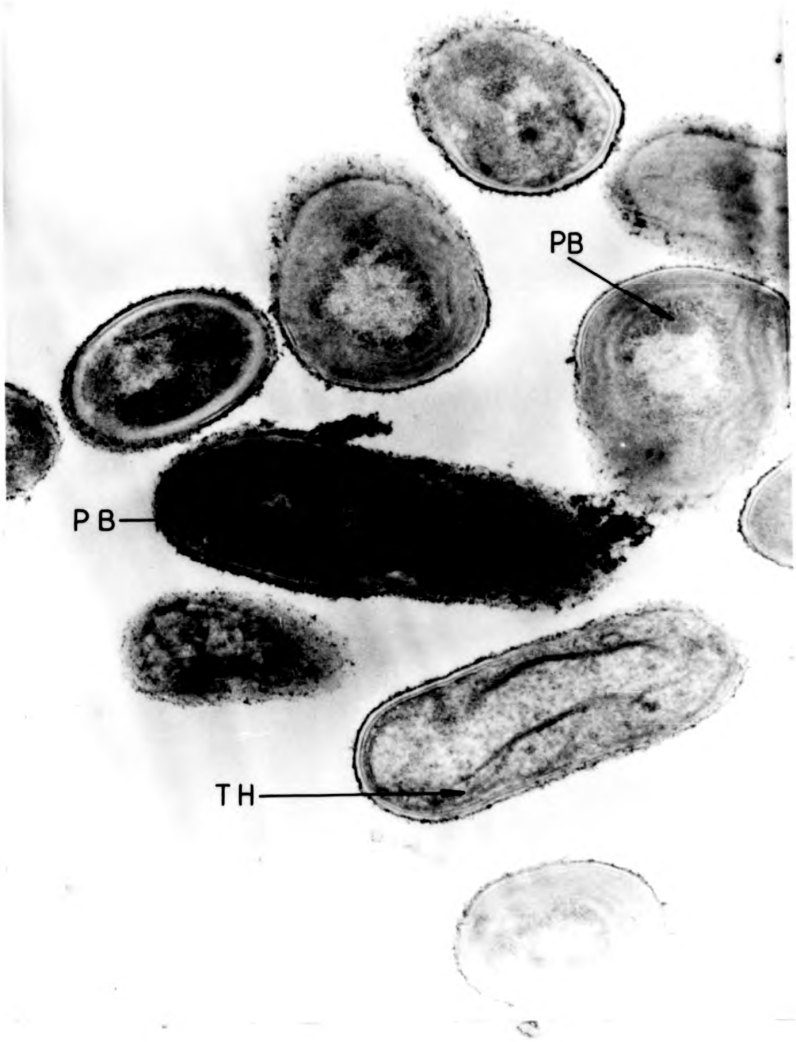

PB
PB
TH

Plate 5.3.

The ultrastructure of *A. nidulans* grown at $D=0.10\ h^{-1}$ in light-limited chemostat culture (X 42,000).

TH, thylakoids; PB, polyhedral body.

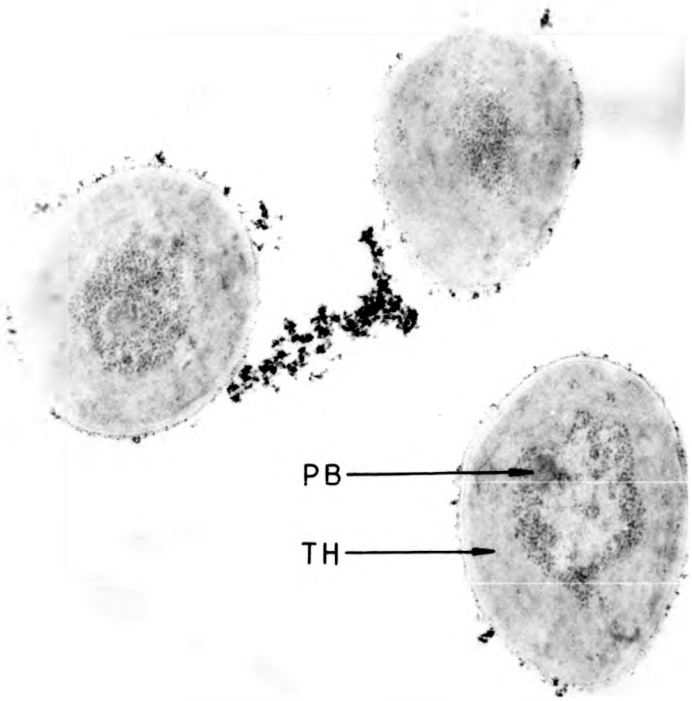
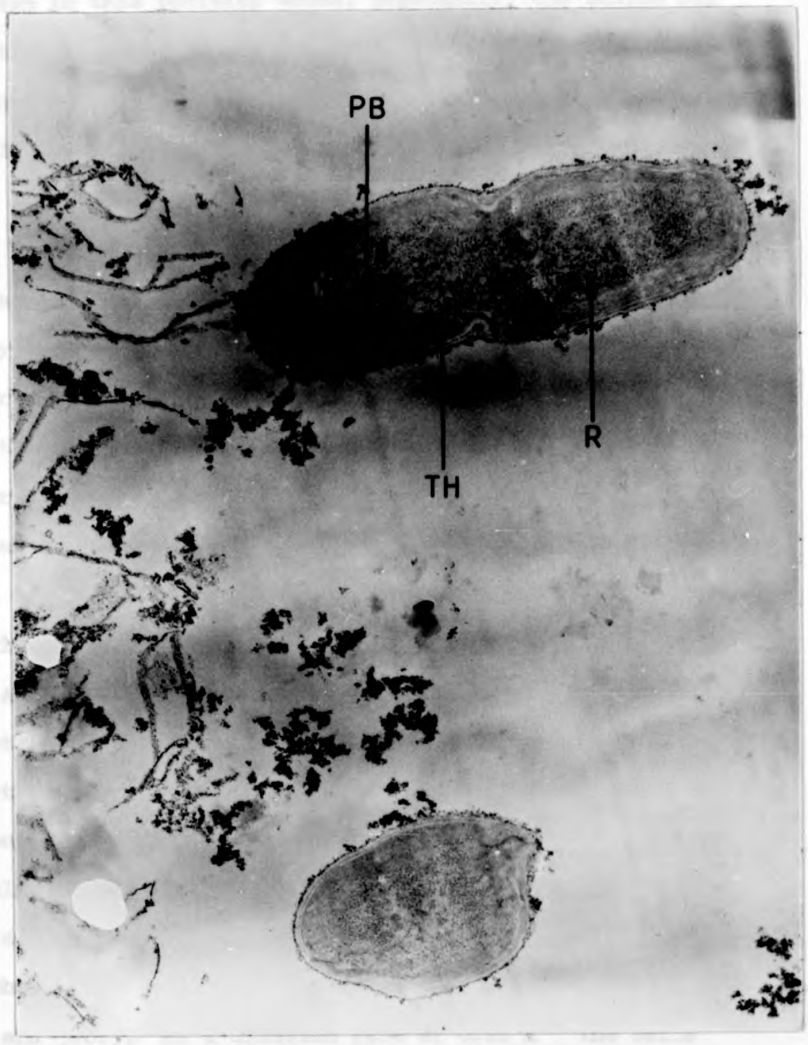

PB
TH

Plate 5.4.

The ultrastructure of *A. nidulans* grown at $D=0.19\ h^{-1}$ in light-limited chemostat culture (X 30,000).

TH, thylakoids; PB, polyhedral body; R, ribosomes.


PB
TH
R


PB
TH
R

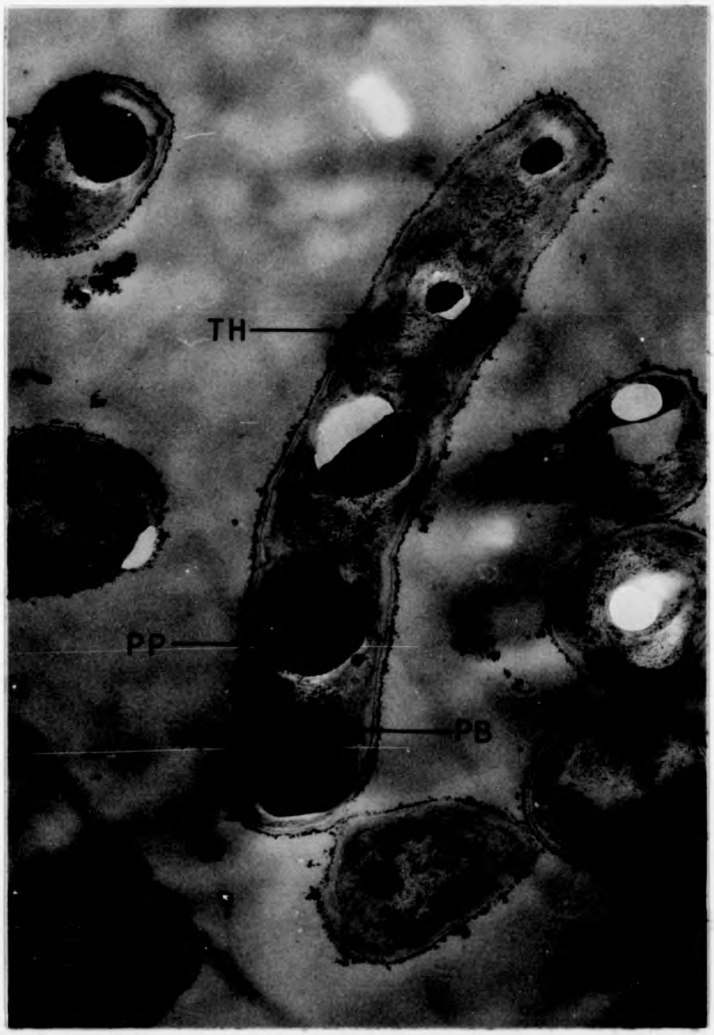
of large densely stained inclusion bodies at a dilution rate of 0.02 h^{-1}. These bodies were located in the central area of the cells (plate 5.5.) although in certain cases a 'hole' was seen in this position where, presumably, the bodies had been torn out of the cell due to sectioning (plate 5.6.). It was suggested that these bodies were large lipid inclusions so a lipid stain (section 2.8.) was carried out on organisms grown at this dilution rate and the cells observed under the light microscope. The results were inconclusive possibly because the cells were so small. However, it seemed more likely that these inclusion bodies were polyphosphate granules rather than lipid due to their positioning in the cell and the fact that according to Fogg et al. (1973) empty areas in electron micrographs of the cytoplasm generally indicated the removal of polyphosphate (section 1.3.7.4.). A metachromatic stain was not, however, carried out so this view cannot be definitely substantiated.

Apart from the presence of these inclusion bodies the fine structure of the organisms grown under carbon dioxide-limiting conditions were similar irrespective of the dilution rate although, as under light-limiting conditions, some of the cellular components did alter quantitatively. At a dilution rate of 0.02 h^{-1} the cells contained in general two to three peripheral thylakoids and up to four polyhedral bodies (plates 5.5. and 5.6.). At a dilution rate of 0.10 h^{-1} the cells contained in general a greater number of peripheral thylakoids,

Plate 5.5.

The ultrastructure of *A. nidulans* grown at $D=0.02\ h^{-1}$ in carbon dioxide-limited chemostat culture (X 30,000).

PP, polyphosphate granule; TH, thylakoids, PB, polyhedral body.


TH
PP
PB

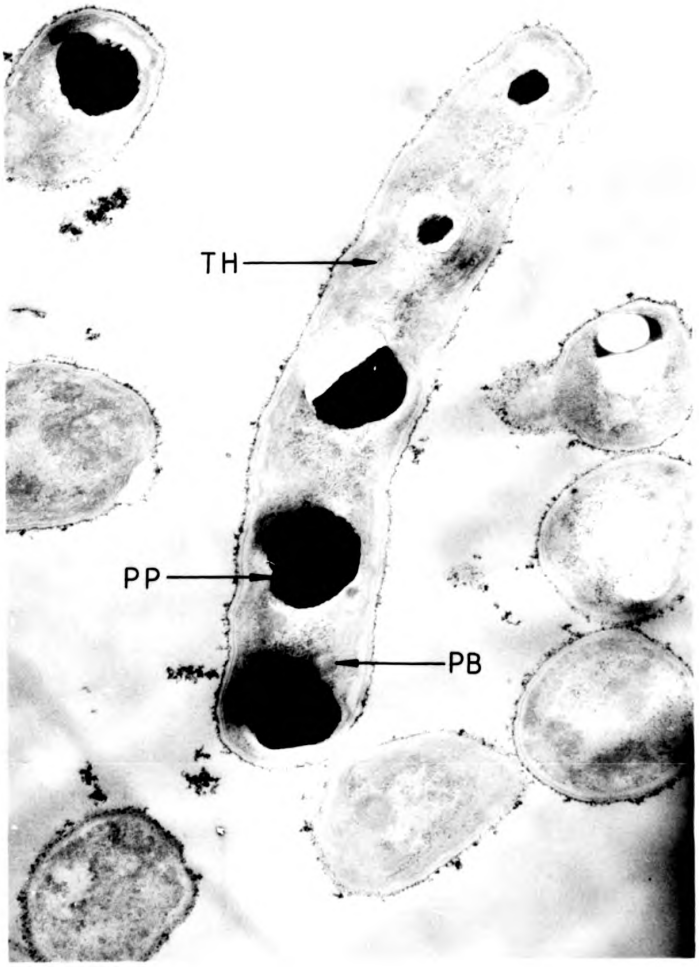
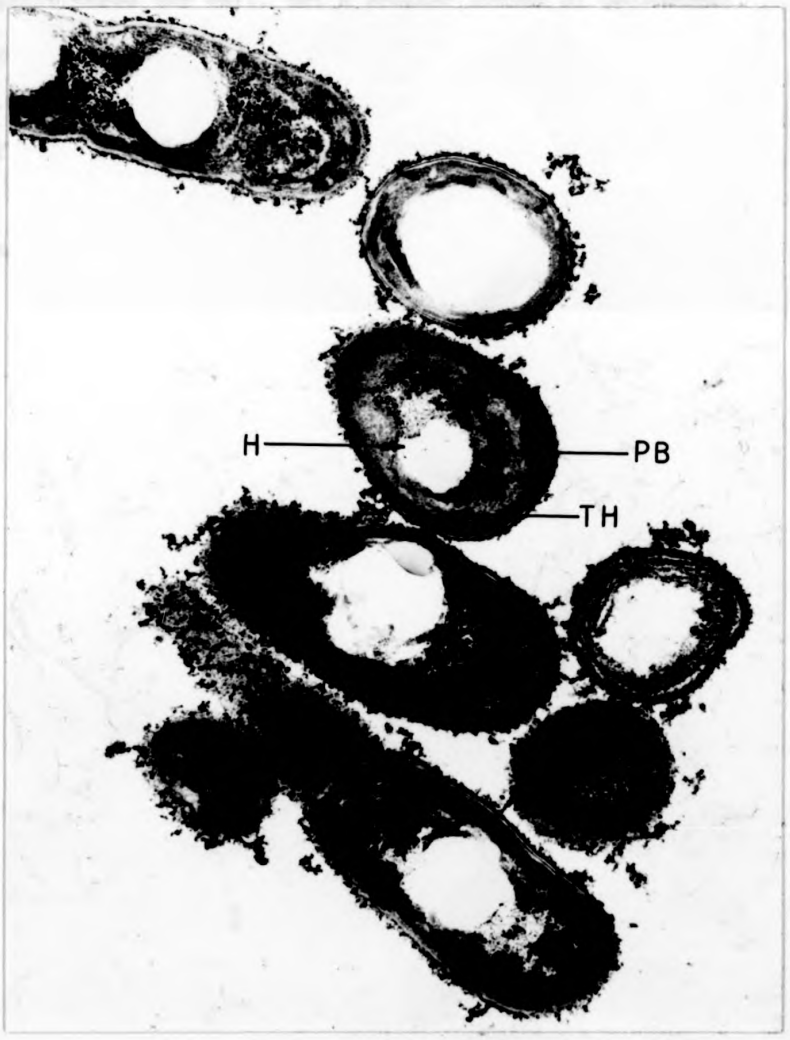
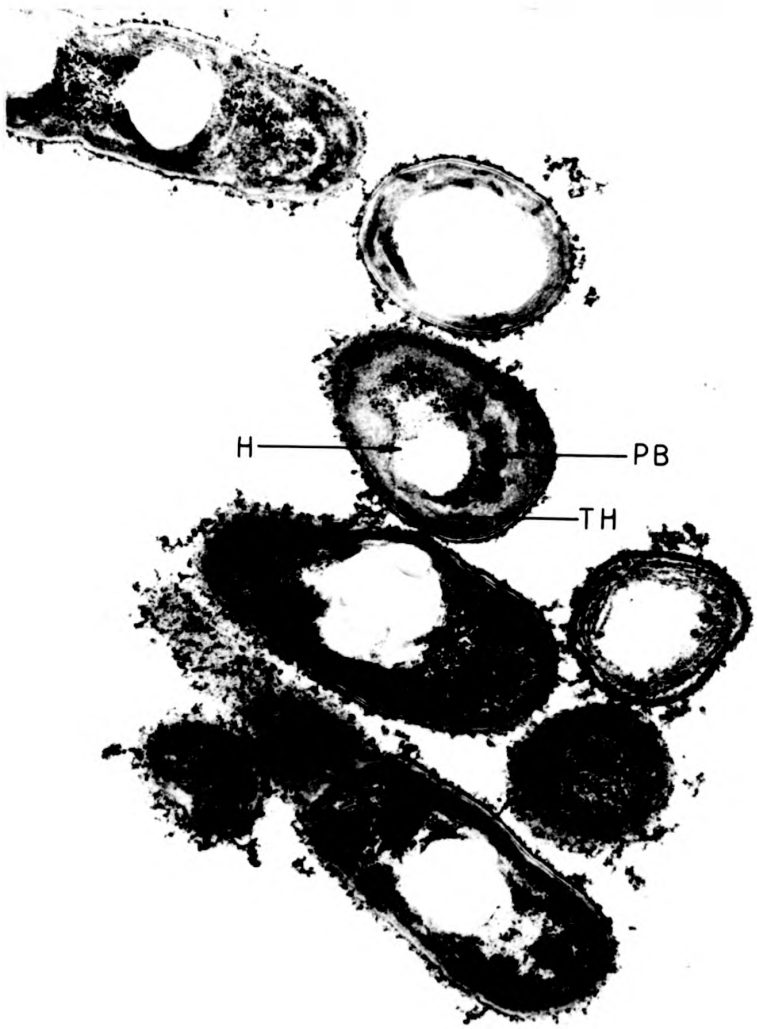

TH
PP
PB

Plate 5.6.

The ultrastructure of A. nidulans grown at D= 0.02 h^{-1} in carbon dioxide-limited chemostat culture (X 36,000).

H, 'hole' due to loss of polyphosphate granule; TH, thylakoids; PB, polyhedral body.


H
PB
TH


H
PB
TH

between three and four, but fewer polyhedral bodies, generally one or two(plate 5.7.). At a dilution rate of 0.19 h^{-1} the cells contained possibly slightly fewer peripheral thylakoids, normally approximately three per cell, but a greater number of polyhedral bodies, generally two or three but sometimes as many as four (plate 5.8.). Also, at this dilution rate the nucleoplasmic region of the cells was consistently more granular in appearance although this was not so marked as under light-limited growth conditions.

Plate 5.7.

The ultrastructure of A. nidulans grown at D=0.10 h^{-1} in carbon dioxide-limited chemostat culture (X 32,000).

TH, thylakoids; PB, polyhedral body.

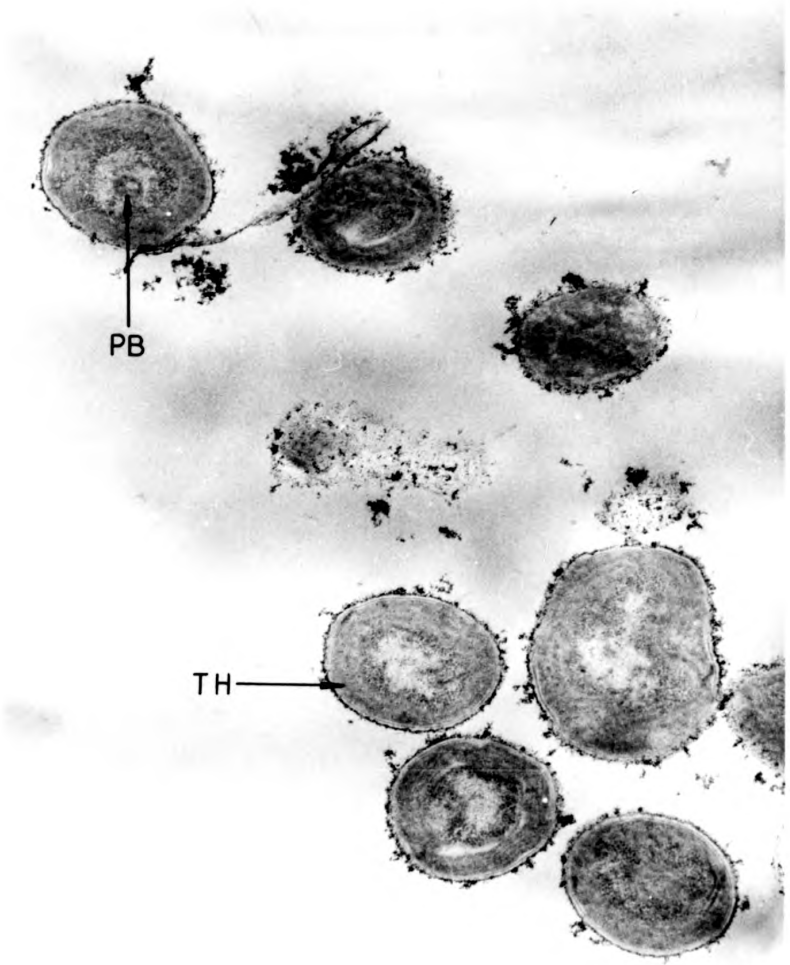
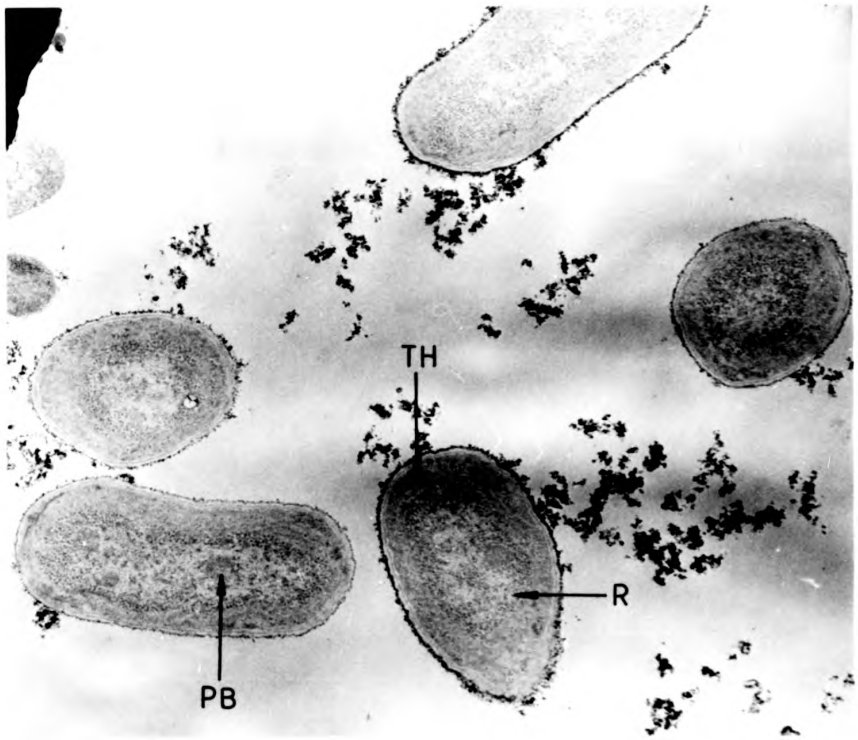

PB
TH

Plate 5.8.

The ultrastructure of A. nidulans grown at D=0.19 h^{-1} in carbon dioxide-limited chemostat culture (X 30,000).

TH, thylakoids; PB, polyhedral body; R, ribosomes.


TH
R
PB

5.4. DISCUSSION

From these results it can be seen that the fine structure of A. nidulans was basically the same irrespective of the growth rate or whether the organisms were grown under light-or carbon dioxide-limiting conditions. The cellular organisation being similar to that previously found for this organism as well as for other cyanobacteria by many other workers (sections 1.3. and 5.1.).

In this study the only significant difference in cellular composition was the large central inclusion bodies found only under carbon dioxide-limiting conditions at D=0.02 h^{-1}. It seemed unlikely that these bodies were composed of lipid due to the inconclusive results obtained using the Sudan black staining procedure (sections 2.8 and 5.3). This view was reinforced by the results obtained for the lipid determinations under the same growth conditions (section 4.5.2. and figure 4.19). Although a slightly higher value was obtained at D=0.02 h^{-1} for the lipid composition of these organisms under carbon dioxide-limiting conditions than expected it still did not seem high enough to account for these large inclusions. Also, these organisms would not be expected to store carbon-containing material under these severely-limiting conditions - the little carbon available being more likely to be used for growth than storage functions. As stated in section 5.3. it seemed more likely that these inclusions were polyphosphate bodies. This would be in agreement with Harold (1966) and Dawes and Senior (1973) who stated that

the deposition of polyphosphate was usually promoted when the growth rate of an organism was low or when its metabolism reflected a nutrient deficiency or imbalance. Both of these factors were relevant in this case. It would also be in agreement with the results of Carr and Sandhu (1966) who found that polyphosphate accumulated when the organisms had a source of ATP formation but lacked carbon for growth. Under the carbon dioxide-limited conditions employed in this study light would have been in excess so that the light reactions of photosynthesis could readily occur leading to ATP formation. However, under the extreme carbon dioxide-limitation at very low dilution rates the dark reactions of carbon dioxide fixation would have been limited so there would presumably have been an excess of ATP produced which could not be used for this process. This excess ATP could therefore have been used to produce polyphosphates, the biosynthesis of which involves the direct utilisation of ATP (Dawes and Senior, 1973). At higher growth rates with more available carbon present for carbon dioxide fixation and presumably more ATP being necessary for nucleic acid metabolism (this antagonistic relationship between polyphosphate and nucleic acid metabolism seeming to be a common phenomenon in various organisms according to Harold (1966)) polyphosphate accumulation would not be expected. Therefore, in agreement with Harold (1966) and Dawes and Senior (1973), polyphosphates would seem to function as a reserve of phosphorus or as a regulator of phosphate economy in this organism rather

than as an energy reserve compound. If these bodies were, in fact, polyphosphate granules this would also help to explain the very low value (63.8%) obtained for the total macromolecular composition of this organism under these growth conditions (section 4.6 and table 4.4.) as this compound would not, of course, have been taken into account by the protein, carbohydrate or lipid determinations . Another indication of the nature of these bodies was the fact that they were not accumulated under light-limited growth conditions. Under these conditions with direct limitation of the light reactions of photosynthesis there would have been a lack of ATP so although carbon dioxide was present in excess fixation would have been limited. Any ATP produced would therefore presumably have been utilised for carbon dioxide fixation rather than for polyphosphate production.

Apart from this difference in the cellular composition of A. nidulans seen only under severe carbon dioxide-limiting growth conditions there were a few quantitative differences in the levels of cellular components according to the growth conditions. For example, the amount of thylakoid membrane under both limitations seemed to be higher at D=0.10 h^{-1} than at D=0.02 h^{-1} but seemed to possibly decrease slightly at D=0.19 h^{-1}. However, this was difficult to determine precisely because although a slight decrease in the number of thylakoids were seen, it was possible that, due to the increase in cell size with increasing dilution rate, a similar amount of thylakoid membrane in total could have been present. Nevertheless, the amount of membrane present seemed

to correlate reasonably well with the pigment composition of this organism (section 4.3.) in agreement with the work of Allen (1968a) on the same organism.

The numbers of polyhedral bodies present in the cells also changed according to the growth conditions. Under carbon dioxide-limiting conditions the numbers were lowest in organisms grown at D=0.10 h^{-1} but increased in organisms grown at both the higher and lower dilution rates. This correlated quite well with the RuBPCase activity at the respective dilution rates (section 6.1.2 and figures 6.3. and 6.4.) so substantiating the view that these bodies contain the RuBPCase enzyme (section 1.3.7.5.). The increased number of bodies in organisms grown at the lowest growth rate under severe carbon dioxide-limitation was in agreement with the results of Beudeker, Cannon, Kuenen and Shively (1980) who found that carbon dioxide-limitation led to a stimulation of carboxysome formation in Thiobacillus neapolitanus. The numbers of carboxysomes found in organisms grown under light-limiting conditions were generally lower than in organisms grown under carbon dioxide-limiting conditions at the same dilution rates. Also, the correlation between carboxysome number and RuBPCase activity (section 6.1.1. and figures 6.1. and 6.2.) was not so good with possibly slightly more carboxysomes being seen in organisms grown at D=0.10 h^{-1} than at D=0.19 h^{-1}. This would perhaps be expected as light-limitation would not presumably affect the activity of the RuBPCase enzyme as directly as the limitation of its substrate, carbon dioxide. The low number

of polyhedral bodies seen in organisms grown at D=0.02 h^{-1} under light-limiting conditions may have been due to the possible nitrogen-limitation under these conditions (section 3.3.) as DeVasconcelos and Fay (1974) found that nitrogen starvation led to a disintegration of polyhedral bodies in Anabaena cylindrica.

Other cellular components which changed in number due to the growth conditions were the ribosomes which seemed to increase with increasing dilution rate under both limitations. As the ribosomes contain the nucleic acid RNA this would be expected from the results of the RNA determinations (section 4.1. and figures 4.2. and 4.6.).

PART 6. CARBOXYLASE DETERMINATIONS FOR ANACYSTIS NIDULANS GROWN UNDER CHEMOSTAT CONTINUOUS-FLOW CULTURE CONDITIONS

The influence of dilution rate on the activities of three carboxylase enzymes, RuBPCase, PEPCase and pyruvate Case, was determined in A. nidulans grown in light-and carbon dioxide-limited chemostat cultures using both the Triton method and the cell-free extract method (section 2.9.).

In all of these determinations a control system was set up with each set of samples to which no substrate was added, with the result that no measurable fixation of [^{14}C] -carbon dioxide occurred, at least during the 20 minutes incubation period of the experiment. Also,it was ensured in each experiment that the amount of incorporation of the sodium [^{14}C] -bicarbonate was proportional to the amount of cell-free extract or cell suspension used. That is, with 0.2 ml extract double the number of counts per minute were obtained as with 0.1 ml extract.

The optimum level of Triton X-100 was found to be 10% (v/v) as a 5% (v/v) solution resulted in significantly less activity of the carboxylase being assayed and a 20% (v/v) solution resulted in slightly less activity. Consequently, a 10% (v/v) solution was used in all appropriate experiments.

6.1. RIBULOSE 1,5-BISPHOSPHATE CARBOXYLASE DETERMINATIONS

6.1.1. Light-limited conditions

The RuBPCase activity, expressed in terms of μmol CO_2 fixed h^{-1} (10^8 organisms)$^{-1}$ and as μmol CO_2 fixed h^{-1} (mg protein)$^{-1}$, was

determined in a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1} using both the Triton method and the cell-free extract method.

Using the Triton method the RuBPCase activity increased approximately 5 to 6-fold with increasing growth rate on both a unit cell and unit protein basis (figure 6.1.). However, different results were obtained for the RuBPCase activity when the cell-free extract assay method was used. In this case the RuBPCase activity increased only 2-fold with increasing growth rate on a unit cell basis and increased only very slightly on a unit protein basis (figure 6.2.). The actual values obtained for RuBPCase activity were higher at the lower growth rates but lower at the higher growth rates than the corresponding values for the Triton method.

6.1.2. Carbon dioxide-limited conditions

The RuBPCase activity, expressed in terms of μmol CO_2 fixed h^{-1} $(10^8$ organisms$)^{-1}$ and as μmol CO_2 fixed h^{-1} (mg protein)$^{-1}$, was determined in a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1} using both the Triton method and the cell-free extract method.

Using the Triton method the RuBPCase activity increased approximately 4 to 5-fold with increasing growth rate from 0.05 to 0.19 h^{-1} on both a unit cell and unit protein basis with a slight increase at the lowest growth rate of approximately 0.02 h^{-1} (figure 6.3.). These results were very similar, although possibly slightly higher, especially at the lowest growth rates,

Figure 6.1.

The influence of dilution rate on the RuBPCase activity, expressed in terms of μmol CO_2 fixed h^{-1} $(10^8$ organisms$)^{-1}$ (O) and as μmol CO_2 fixed h^{-1} (mg protein)$^{-1}$ (●), of *A. nidulans* grown in light-limited chemostat culture as determined by the Triton assay method.

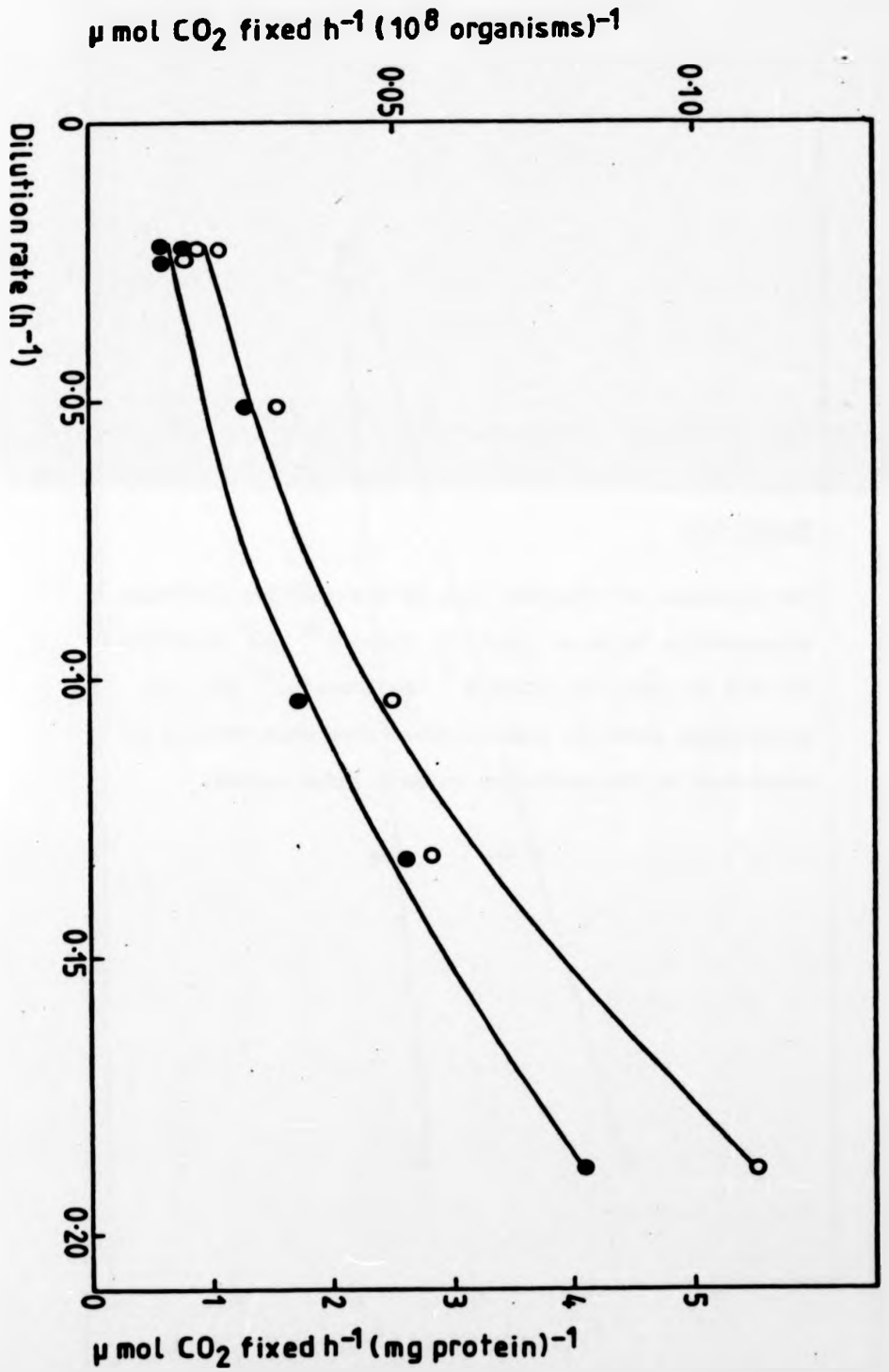

μ mol CO_2 fixed h^{-1} (10^8 organisms)$^{-1}$
0·05
0·10
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0
1
2
3
4
5
μ mol CO_2 fixed h^{-1} (mg protein)$^{-1}$

Figure 6.2.

The influence of dilution rate on the RuBPCase activity, expressed in terms of μmol CO_2 fixed h^{-1} $(10^8$ organisms$)^{-1}$ (○) and as μmol CO_2 fixed h^{-1} (mg protein)$^{-1}$ (●), of A. nidulans grown in light-limited chemostat culture as determined by the cell-free extract assay method.

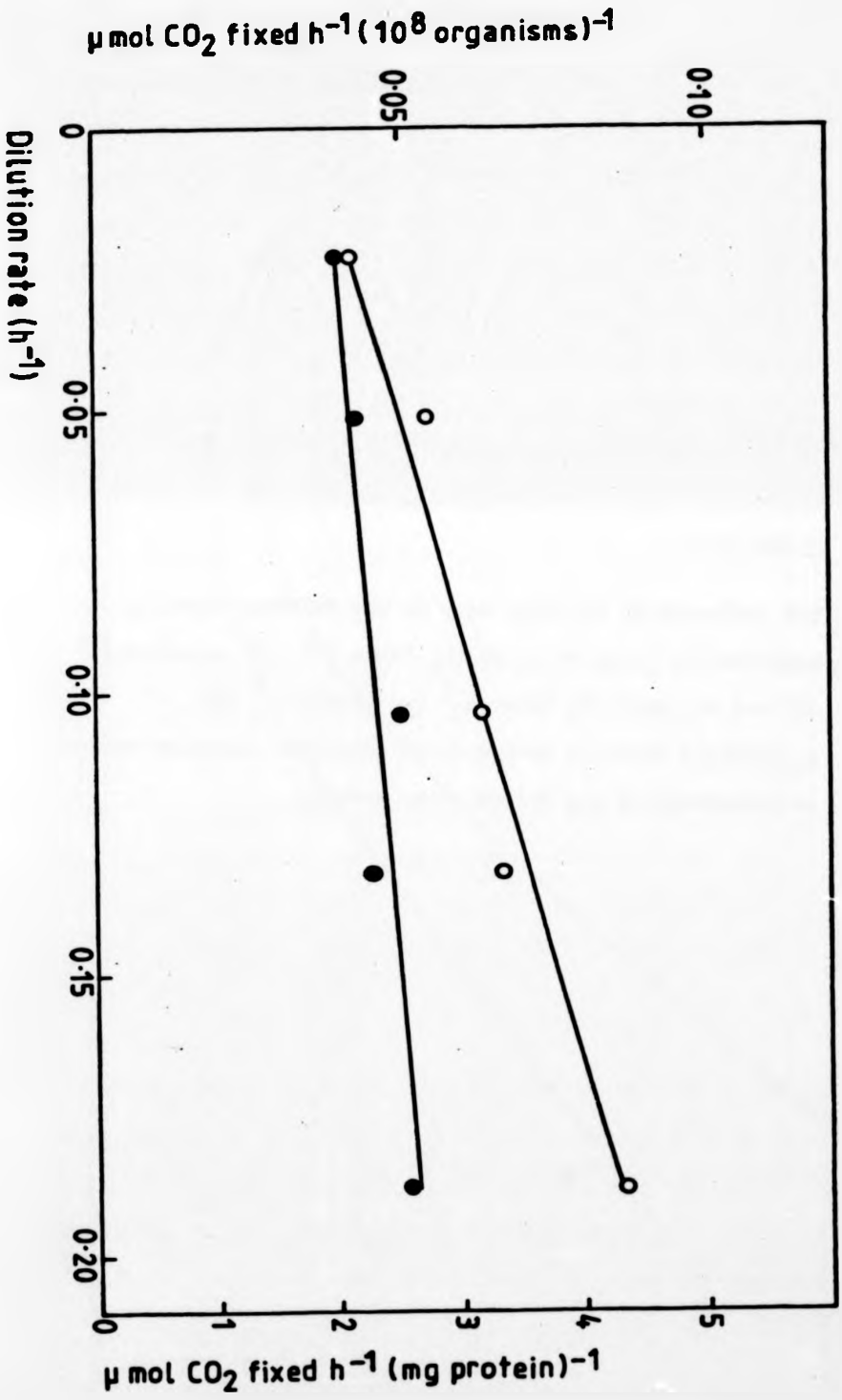
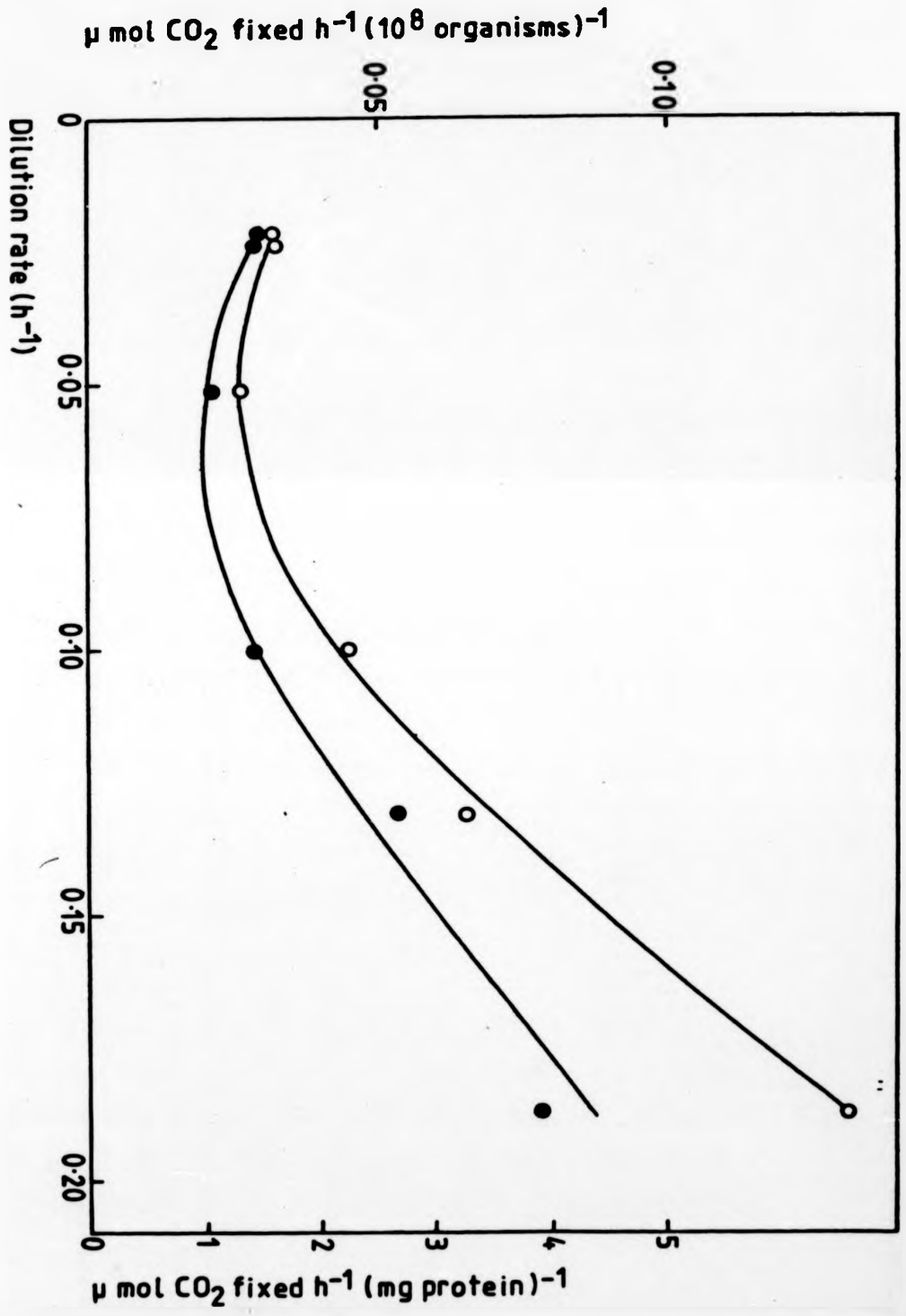

μ mol CO_2 fixed h^{-1} (10^8 organisms)$^{-1}$
0·05
0·10
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
1
2
3
4
5
μ mol CO_2 fixed h^{-1} (mg protein)$^{-1}$

Figure 6.3.

The influence of dilution rate on the RuBPCase activity, expressed in terms of μmol CO_2 fixed h^{-1} (10^8 organisms)$^{-1}$ (○) and as μmol CO_2 fixed h^{-1} (mg protein)$^{-1}$ (●), of *A. nidulans* grown in carbon dioxide-limited chemostat culture as determined by the Triton assay method.


μ mol CO_2 fixed h^{-1} (10^8 organisms)$^{-1}$
0·05
0·10
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
1
2
3
4
5
μ mol CO_2 fixed h^{-1} (mg protein)$^{-1}$

to the corresponding RuBPCase activities shown under light-limited growth conditions using the Triton method of determination.

These results were also similar although again generally higher to those obtained for RuBPCase activity using the cell-free extract method. In this case the RuBPCase activity increased approximately 3 to 4-fold with increasing growth rate from 0.05 to 0.19 h^{-1} on both a unit cell and unit protein basis with a greater increase in activity at the lowest growth rate of approximately 0.02 h^{-1} than shown by the Triton method (figure 6.4.). These results differed considerably from the corresponding RuBPCase activities shown using the same method of determination under light-limited growth conditions.

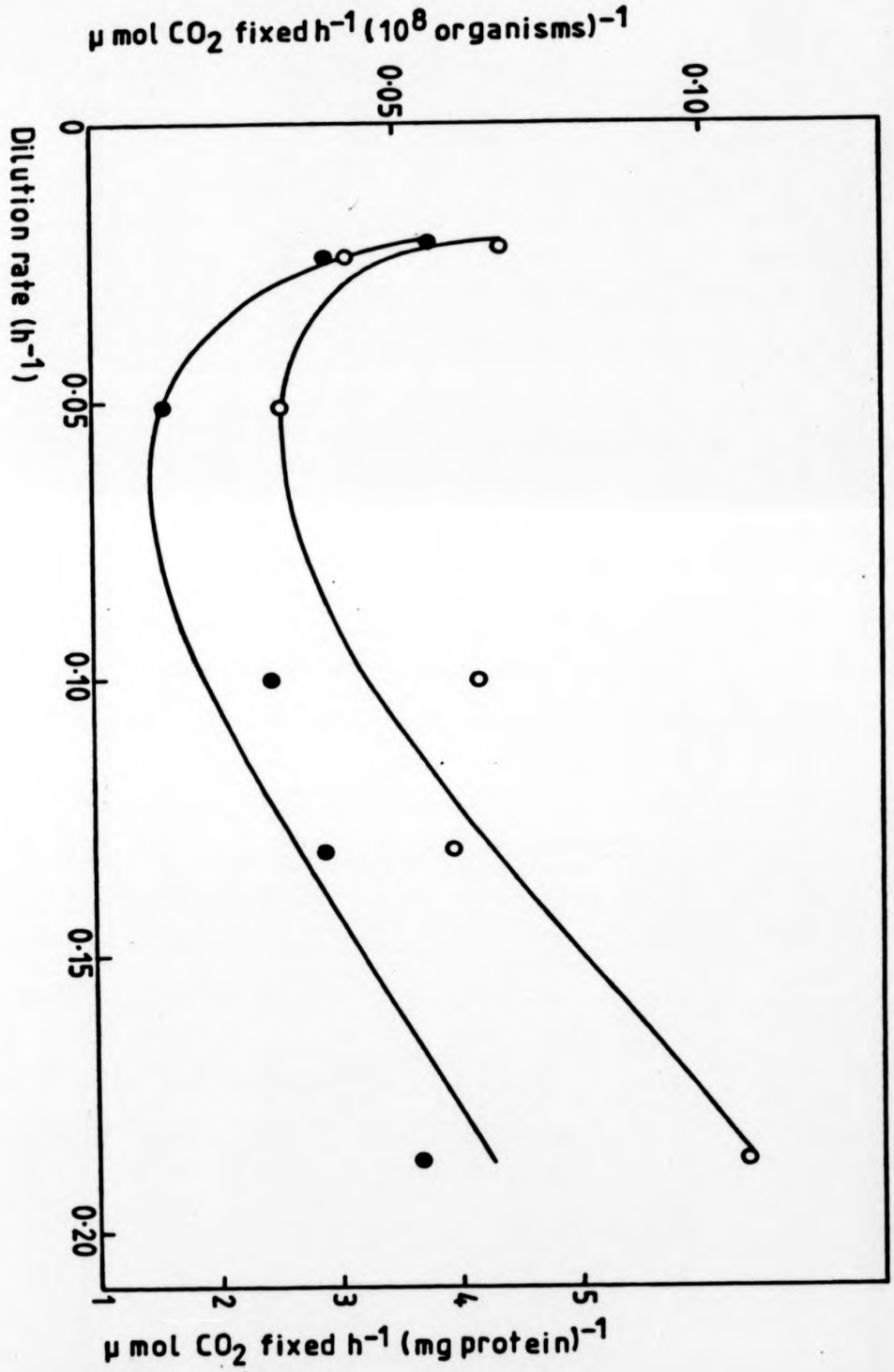
6.2. PHOSPHOENOL PYRUVATE CARBOXYLASE DETERMINATIONS

Acetyl-CoA was found to have no effect on the activity of PEPCase which was in agreement with the results of Codd and Stewart (1973) for Anabaena cylindrica. On the other hand, the presence of glutamate oxaloacetic transaminase was necessary for maximum activity. This was used to convert the unstable product of the PEPCase reaction, oxaloacetate, to aspartate (Glover and Morris, 1979).

Both methods of cell disruption were used in these determinations although PEPCase activity was only shown by the use of the Triton method. No activity was shown under any of the growth conditions when the cell-free extract method was used. In this case the activity may have been lost due to the severity of the cell breakage and the use of Tris-HCl buffer as suggested by Colman,

Figure 6.4.

The influence of dilution rate on the RuBPCase activity, expressed in terms of μmol CO_2 fixed h^{-1} $(10^8$ organisms$)^{-1}$ (○) and as μmol CO_2 fixed h^{-1} (mg protein)$^{-1}$ (●), of A. nidulans grown in carbon dioxide-limited chemostat culture as determined by the cell-free extract assay method.


μ mol CO_2 fixed h^{-1} (10^8 organisms)$^{-1}$
0·05
0·10
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
1
2
3
4
5
μ mol CO_2 fixed h^{-1} (mg protein)$^{-1}$

Cheng and Ingle (1976) or, less likely, the PEPCase may have been lost with the cell debris on centrifugation.

6.2.1. Light-limited conditions

The PEPCase activity, expressed in terms of μmol CO_2 fixed h^{-1} $(10^8$ organisms$)^{-1}$ and as μmol CO_2 fixed h^{-1} (mg protein$)^{-1}$, was determined in a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

The PEPCase activity decreased approximately 3 to 4-fold with increasing growth rate on both a unit cell and unit protein basis although it seemed to level off showing a plateau region from growth rates of approximately 0.07 h^{-1} to 0.13 h^{-1} (figure 6.5.).

6.2.2. Carbon dioxide-limited conditions

The PEPCase activity, expressed in terms of μmol CO_2 fixed h^{-1} $(10^8$ organisms$)^{-1}$ and as μmol CO_2 fixed h^{-1} (mg protein$)^{-1}$, was determined in a number of steady state cultures over the dilution rate range, D=0.02 to 0.19 h^{-1}.

Although the pattern of PEPCase activity differed from that obtained under light-limited conditions, the PEPCase still showed a decrease in activity with increasing growth rate on both a unit cell and unit protein basis (figure 6.6.). In general the values were higher than those obtained for the corresponding growth rates under light-limitation.

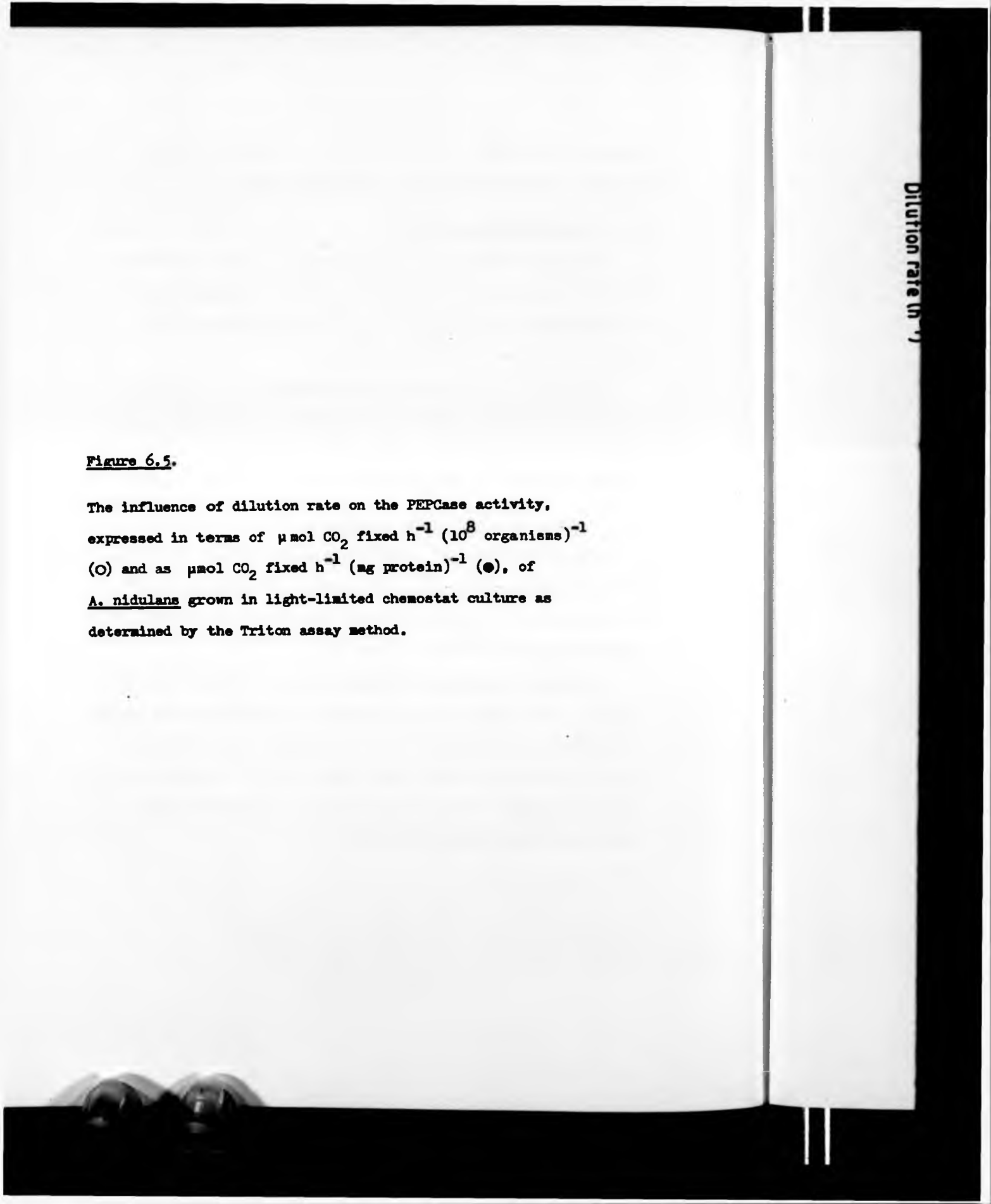
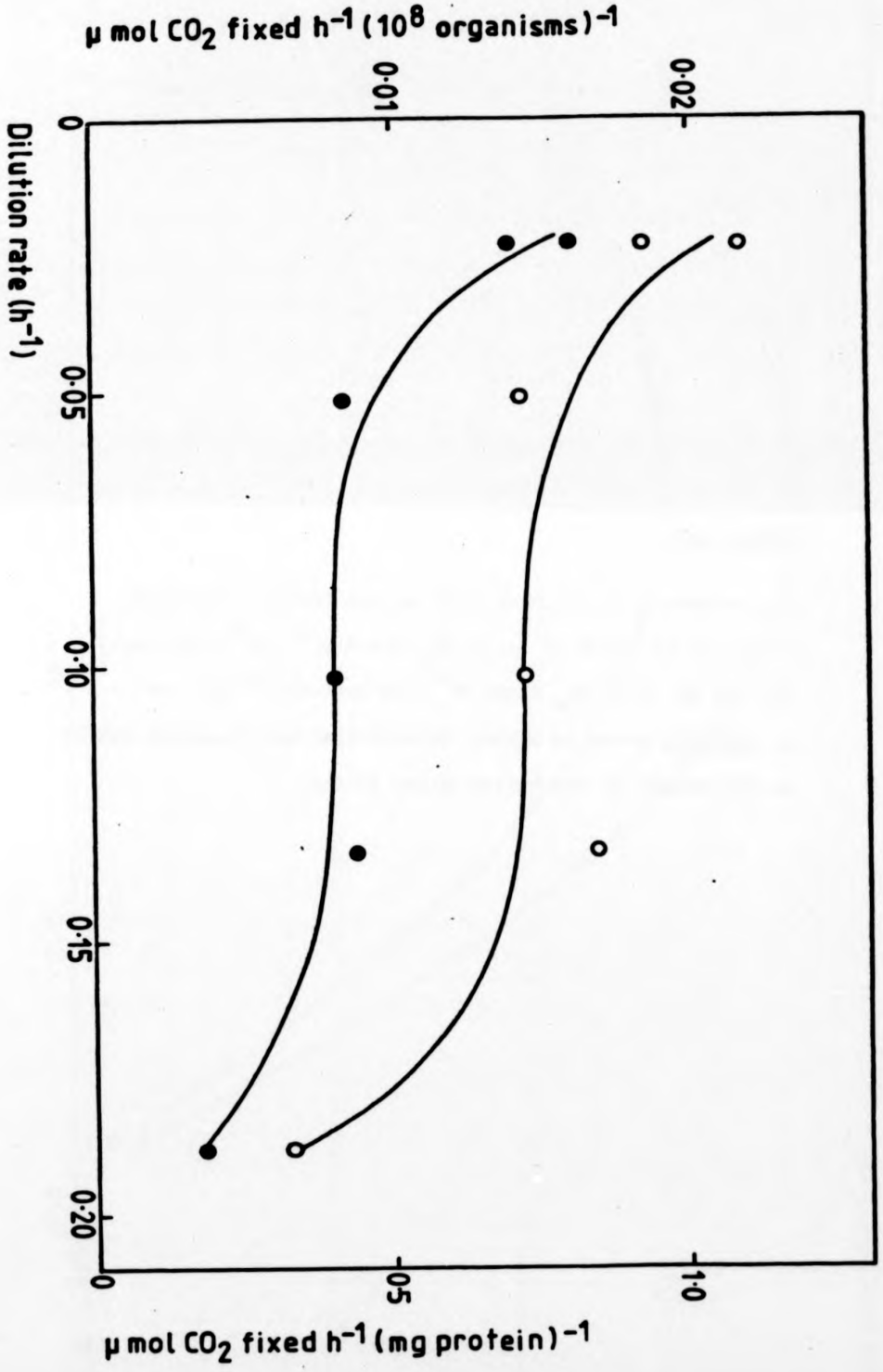


Figure 6.5.

The influence of dilution rate on the PEPCase activity, expressed in terms of μmol CO_2 fixed h^{-1} (10^8 organisms)$^{-1}$ (○) and as μmol CO_2 fixed h^{-1} (mg protein)$^{-1}$ (●), of *A. nidulans* grown in light-limited chemostat culture as determined by the Triton assay method.


μ mol CO_2 fixed h^{-1} (10^8 organisms)$^{-1}$
0·01
0·02
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0
0·5
1·0
μ mol CO_2 fixed h^{-1} (mg protein)$^{-1}$

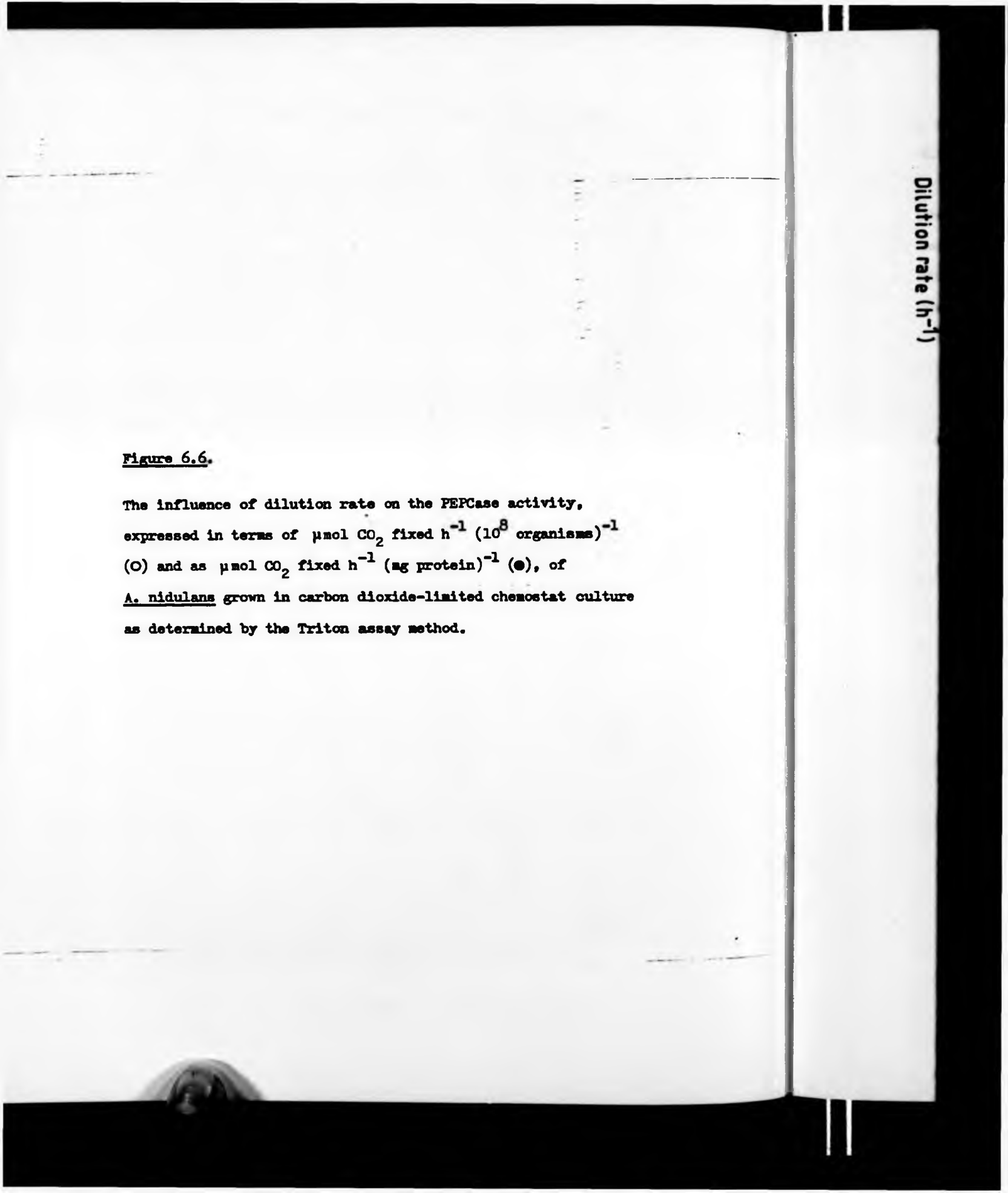
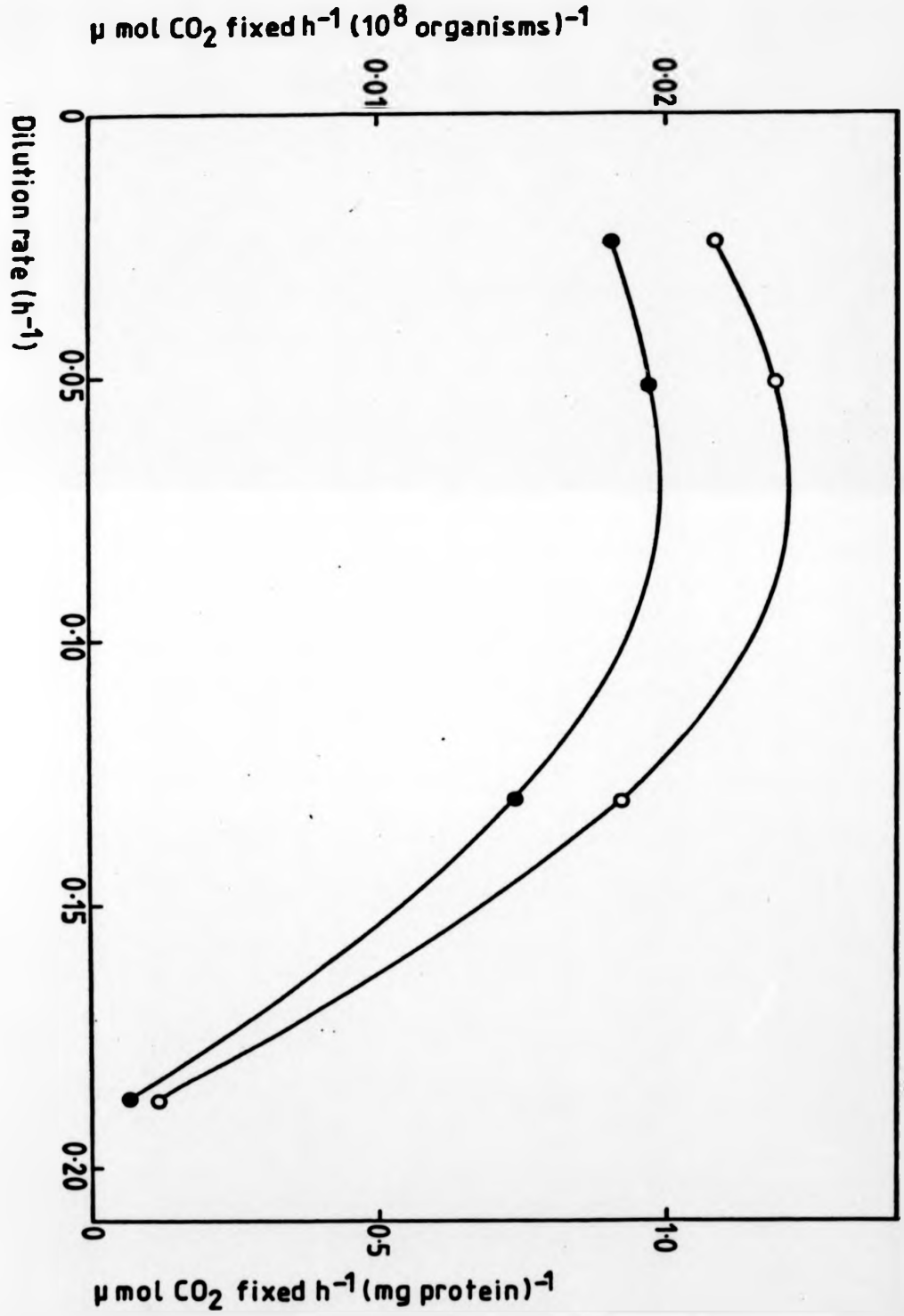


Figure 6.6.

The influence of dilution rate on the PEPCase activity, expressed in terms of μmol CO_2 fixed h^{-1} (10^8 organisms)$^{-1}$ (○) and as μmol CO_2 fixed h^{-1} (mg protein)$^{-1}$ (●), of *A. nidulans* grown in carbon dioxide-limited chemostat culture as determined by the Triton assay method.


μ mol CO_2 fixed h^{-1} (10^8 organisms)$^{-1}$
0·01
0·02
Dilution rate (h^{-1})
0
0·05
0·10
0·15
0·20
0
0·5
1·0
μ mol CO_2 fixed h^{-1} (mg protein)$^{-1}$

6.3. PYRUVATE CARBOXYLASE DETERMINATIONS

No pyruvate carboxylase activity was obtained under any of the growth conditions used with either the Triton or cell-free extract assay methods in the presence or absence of acetyl-CoA. This was in agreement with the results of Codd and Stewart (1973) who were unable to detect pyruvate Case activity in A. cylindrica.

6.4. DISCUSSION

These results showed that growth rate in particular had a significant effect on the RuBPCase activity of *A. nidulans*, a phenomenon also found to be characteristic of many of the macromolecular components of this organism (section 4.). The actual values obtained for RuBPCase activity on both a per cell and per unit protein basis under most growth conditions were comparable at similar growth rates under both light- (except when the cell-free extract method was used) and carbon dioxide-limitation. An exception was found at very low growth rates under carbon dioxide-limiting conditions when a definite increase in RuBPCase activity was seen. This was in agreement with the work of Kuenen and Veldkamp (1973) and Beudeker, Cannon, Kuenen and Shively (1980) on *Thiobacillus neapolitanus* and Karagouni (1979) and Karagouni and Slater (1979) on *A. nidulans* who found that RuBPCase activity increased due to carbon dioxide-limitation. The kinetics of conventional nutrient-limited chemostat culture predicts that the carbon dioxide concentration would decrease with decreasing dilution rate so that extremely low levels of available carbon dioxide would be expected at the low dilution rates under these limiting conditions. In such an environment an increased RuBPCase activity would be an advantage resulting in a greater capacity to scavenge for and assimilate the low, growth-limiting concentrations of the sole carbon source so resulting in an increased rate of carbon dioxide fixation. This principal of an elevated activity of an initial enzyme involved in the metabolism of a growth-limiting nutrient is, in fact, well established (Dean, 1972).

The high RuBPCase levels at the lowest growth rate (approximately 0.02 h^{-1})under carbon dioxide-limitation was found to correlate with a high number of carboxysomes in the cells of this organism as seen under the electron microscope (section 5.3.). This is also in agreement with the work of Beudeker et al. (1980) who found an increased number of carboxysomes in the cells of T. neapolitanus due to carbon dioxide-limitation. In addition the numbers of carboxysomes in carbon dioxide-limited cells of A. nidulans at higher growth rates seemed to correlate with the RuBPCase activity with fewer carboxysomes present at 0.10 h^{-1} but an increase in number at 0.19 h^{-1} (section 5.3.). However, there was not such a good correlation with RuBPCase activity shown in light-limited organisms with the greatest number of carboxysomes being seen at 0.10 h^{-1} (section 5.2.). In addition there was no correlation of RuBPCase activity shown under either limitation with the pattern of carbon dioxide fixation as determined by Karagouni (1979) for this organism under these conditions. This being in agreement with the results of Karagouni (1979), Karagouni and Slater (1979) and Beudeker et al. (1980) and emphasizes the problems of correlating enzyme activity with the behaviour of intact organisms.

In this study an increase in RuBPCase activity was generally shown with increasing growth rate which would be expected due to the greater requirement of fast growing organisms to generate cellular carbon. However, this was not in agreement with the results of Karagouni (1979) and Karagouni and Slater (1979) on

the same organism which showed a levelling off of RuBPCase activity at the higher growth rates. This may have been due to the fact that the enzyme assays were carried out at 30°C rather than at the growth temperature of 40°C as these authors also showed that temperature affected RuBPCase activity and it is possible that lowering of the temperature would affect the fastest growing cells most markedly.

The two different assay methods used in this study also gave different results. This was obviously most marked in the case of PEPCase as no activity at all was shown after disruption using the French pressure cell and to a lesser extent for the RuBPCase activity under light-limited conditions. This shows the necessity for careful planning when enzyme assays are undertaken.

The activity of PEPCase tended to show an inverse correlation with that of RuBPCase which was especially evident under carbon dioxide-limiting conditions. At most growth rates under both limitations the PEPCase activity was significantly lower than that of RuBPCase with the exception of the lowest growth rates when similar activities were obtained. This was in agreement with the results of Ihlenfeldt and Gibson (1975a), Benedict (1978) and Glover and Morris (1979) who reported that PEPCase activity would be expected to be more significant at low carbon dioxide concentrations and when the growth and photosynthetic rate was low. From these results it seems that the C_4 pathway could be significant in A. nidulans although only at low growth rates

under the conditions examined. However, it is not known whether the PEPCase was actually active in a C_4 photosynthetic pathway comparable with that found in certain higher plants or whether, as suggested by Weathers and Allen (1978), it was acting to compensate for the incomplete tricarboxylic acid cycle. The presence or absence of PEP synthetase was not determined so these results do not give conclusive evidence for or against the operation of the C_4 pathway in A. nidulans under these growth conditions.

No pyruvate carboxylase activity was found under any of the growth conditions examined which was in agreement with the results for A. cylindrica (Codd and Stewart, 1973). The lack of this enzyme tending to indicate that the PEPCase would be required to catalyse the formation of oxaloacetate which could be fed into the tricarboxylic acid cycle.

This study on the carboxylase enzymes of A. nidulans showed that the organism was capable of altering the specific activities of RuBPCase and PEPCase in different ways according to the environmental conditions. In addition the same enzyme behaved differently under light-and carbon dioxide-limitation as well as differences in the specific activities due to changing growth rate. These variations between the two enzymes showed that changes in the specific activities were not associated with a general phenomenon, such as an alteration in the total protein content of the organism, since such changes would have been expected to alter all activities in a similar manner. Therefore, these results suggest that these enzymes might be under transcriptional

control in A. nidulans, in agreement with the results obtained for RuBPCase activity in this organism by Karagouni and Slater (1979), although it is possible that regulation could be at the translational level or at the enzyme level due to activation or changes in the rates of enzyme turnover.

Transcriptional control in cyanobacteria does seem possible despite the proposal of Carr (1973a) that these organisms do not in general regulate their metabolism at the level of gene expression. According to Stanier and Cohen-Bazire (1977) it is hard to imagine that any free-living microbial group could survive if its members were totally unable to regulate gene expression. In fact, a number of proteins are now known to be subject to induction or repression in cyanobacteria with control probably being exerted at the transcriptional level although this has not been shown directly, for example, glucose-6-phosphate dehydrogenase (Raboy, Padan and Shilo, 1976), glycogen phosphorylase (Singer and Doolittle, 1975), ferredoxin (Hutber, Hutson and Rogers, 1977), alkaline phosphatase (Bone, 1971a; Ihlenfeldt and Gibson, 1975b), nitrogenase (Bone, 1971b) and RuBPCase (Karagouni, 1979; Karagouni and Slater, 1979).

PART 7. COMPETITION STUDIES OF ANACYSTIS NIDULANS AND THE GREEN ALGA SCENEDESMUS QUADRICAUDA GROWN UNDER CHEMOSTAT CONTINUOUS-FLOW CULTURE CONDITIONS

In these studies the organisms were grown in light-and carbon dioxide-limited chemostat culture (section 2.10.2.) under aerobic conditions (as green algae require aerobic conditions for growth) and at a temperature of 27°C (as this was about the optimum temperature for the growth of S. quadricauda and this organism could not survive at 40°C, the optimum temperature for the growth of A. nidulans) (section 2.10.1.). Under light-limited conditions the growth medium used was Medium C plus thiamine (as determined in section 2.10.1) which was supplemented with $NaHCO_3$ under carbon dioxide-limited conditions (section 2.10.2.).

7.1. BASIC GROWTH CHARACTERISTICS UNDER LIGHT-LIMITED CONDITIONS

The basic growth characteristics, in terms of absorbance, dry weight and cell number, of both A. nidulans and S. quadricauda grown axenically were determined in a number of steady state cultures under light-limited conditions over an appropriate growth rate range after the maximum specific growth rate (μ_{max}) had been determined from batch growth curves. These basic growth characteristics were compared with the results obtained previously under light-limited conditions (section 3.3.).

7.1.1. A. nidulans

The μ_{max} for A. nidulans grown under batch growth conditions was 0.065 h^{-1} although it was impossible to obtain perfect steady state cultures under continuous-flow culture conditions at

dilution rates above 0.05 h^{-1}. Therefore, the culture biomass, expressed in terms of absorbance, dry weight (mg ml^{-1}) and cell number (organisms ml^{-1}), was determined in a number of steady state cultures over the dilution rate range, D=0.018 to 0.050 h^{-1}.

The biomass concentration, measured in terms of absorbance, dry weight and cell number, decreased rapidly with increasing dilution rate over the growth rate range studied (figure 7.1.). These basic growth characteristics were similar to those found previously under light-limited continuous-flow growth conditions (section 3.3.) although in this case the growth conditions were obviously not optimal for this organism as steady state growth rates above 0.05 h^{-1} could not be obtained. This was presumably due to the lower temperature used for these studies and the aerobic growth conditions.

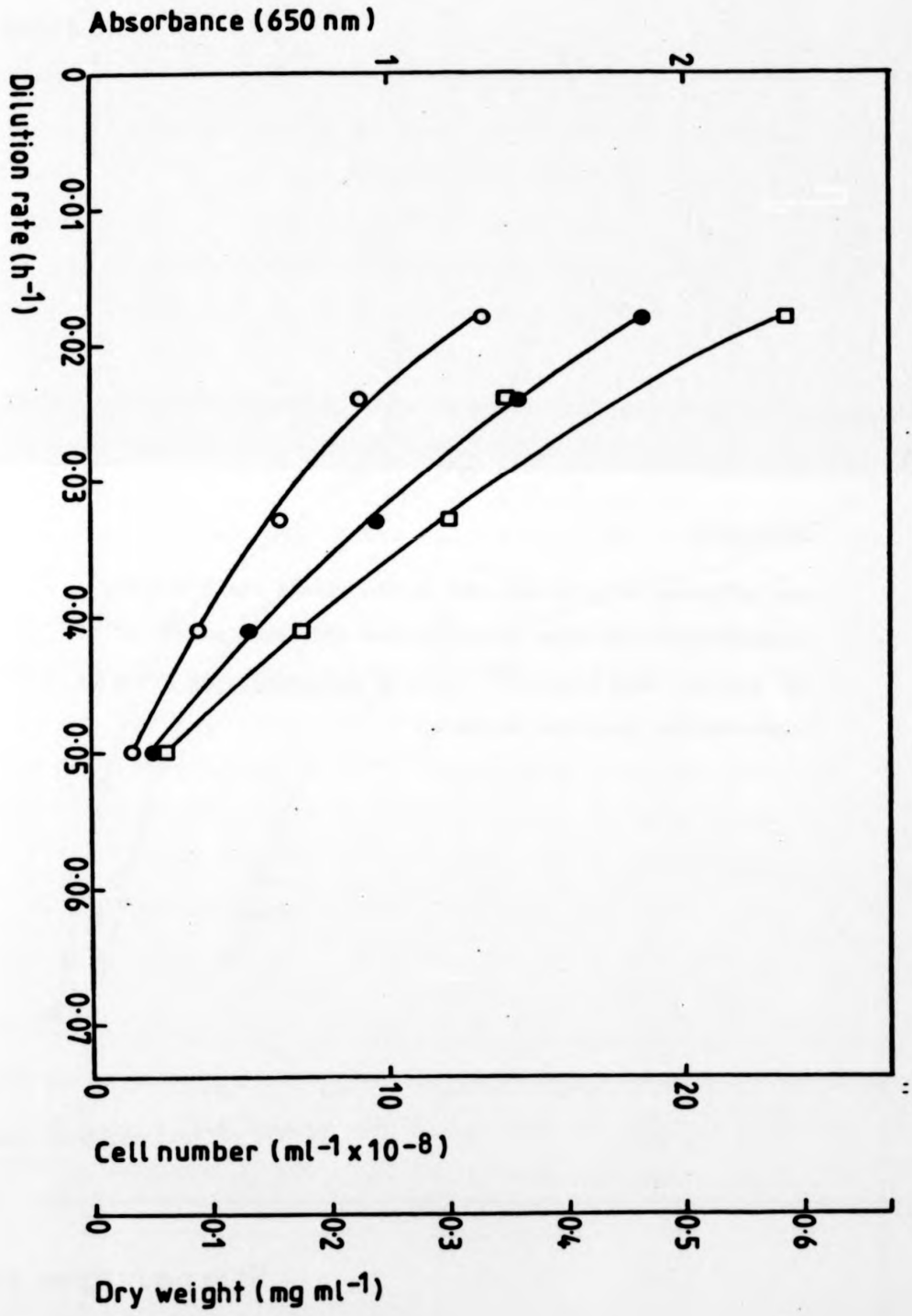
7.1.2. S. quadricauda

The μ_{max} for S. quadricauda grown under batch growth conditions was 0.071 h^{-1}. Therefore, the culture biomass, expressed in terms of absorbance, dry weight (mg ml^{-1}) and cell number (organisms ml^{-1}), was determined in a number of steady state cultures over the dilution rate range, D=0.019 to 0.069 h^{-1}.

The biomass concentration, measured in terms of absorbance, dry weight and cell number, decreased rapidly with increasing dilution rate over the growth rate range studied (figure 7.2.). These basic growth characteristics were similar to those found for A. nidulans under the same light-limited continuous-flow growth conditions (section 7.1.1.).

Figure 7.1.

The influence of dilution rate on the steady state biomass concentration, in terms of absorbance (●), cell number ml^{-1} (○) and dry weight (mg ml^{-1}) (□), of A. nidulans grown in light-limited chemostat culture.


Absorbance (650 nm)
0
1
2
Dilution rate (h⁻¹)
0·01
0·02
0·03
0·04
0·05
0·06
0·07
0
10
20
Cell number (ml⁻¹ x 10⁻⁸)
0
0·1
0·2
0·3
0·4
0·5
0·6
Dry weight (mg ml⁻¹)

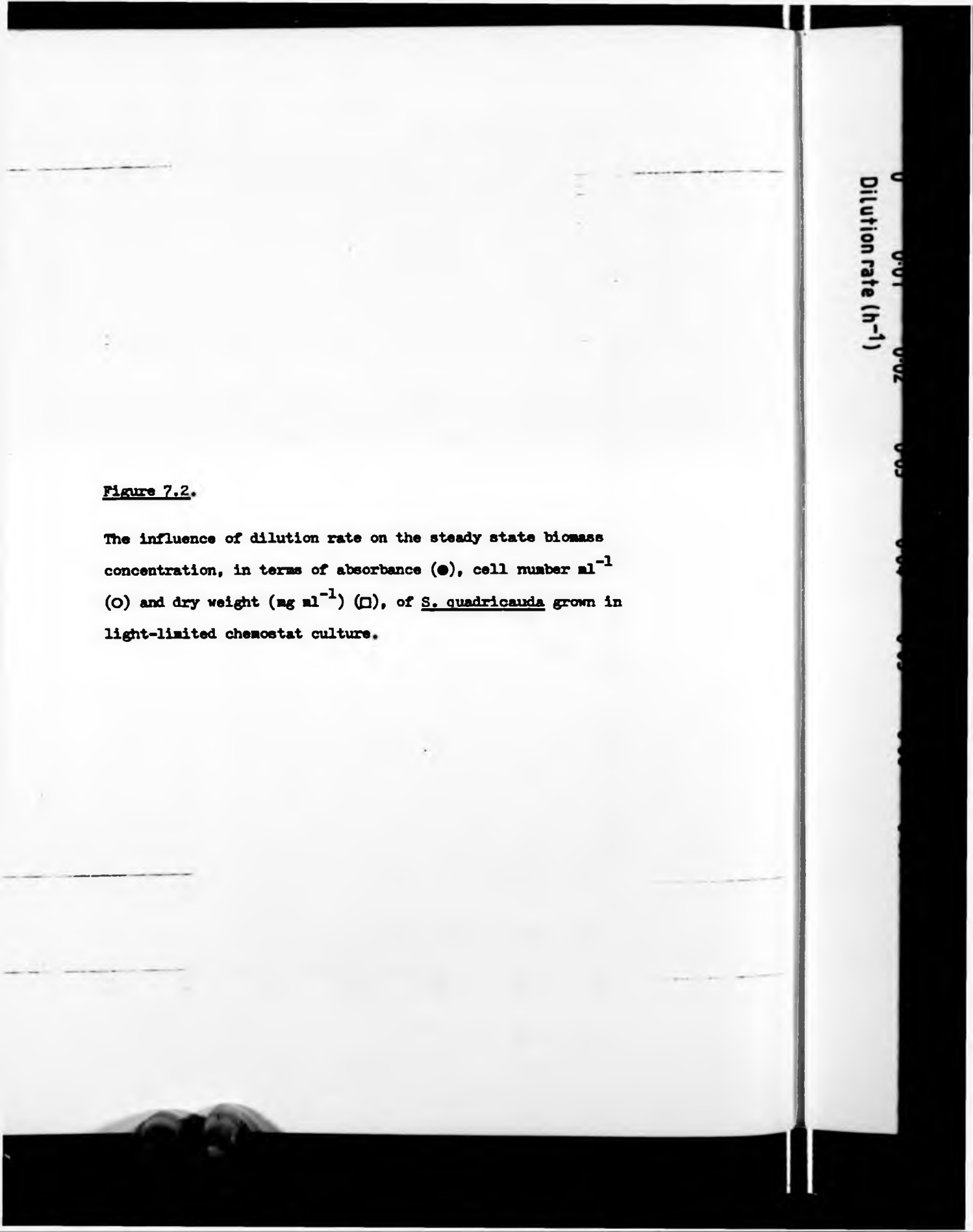
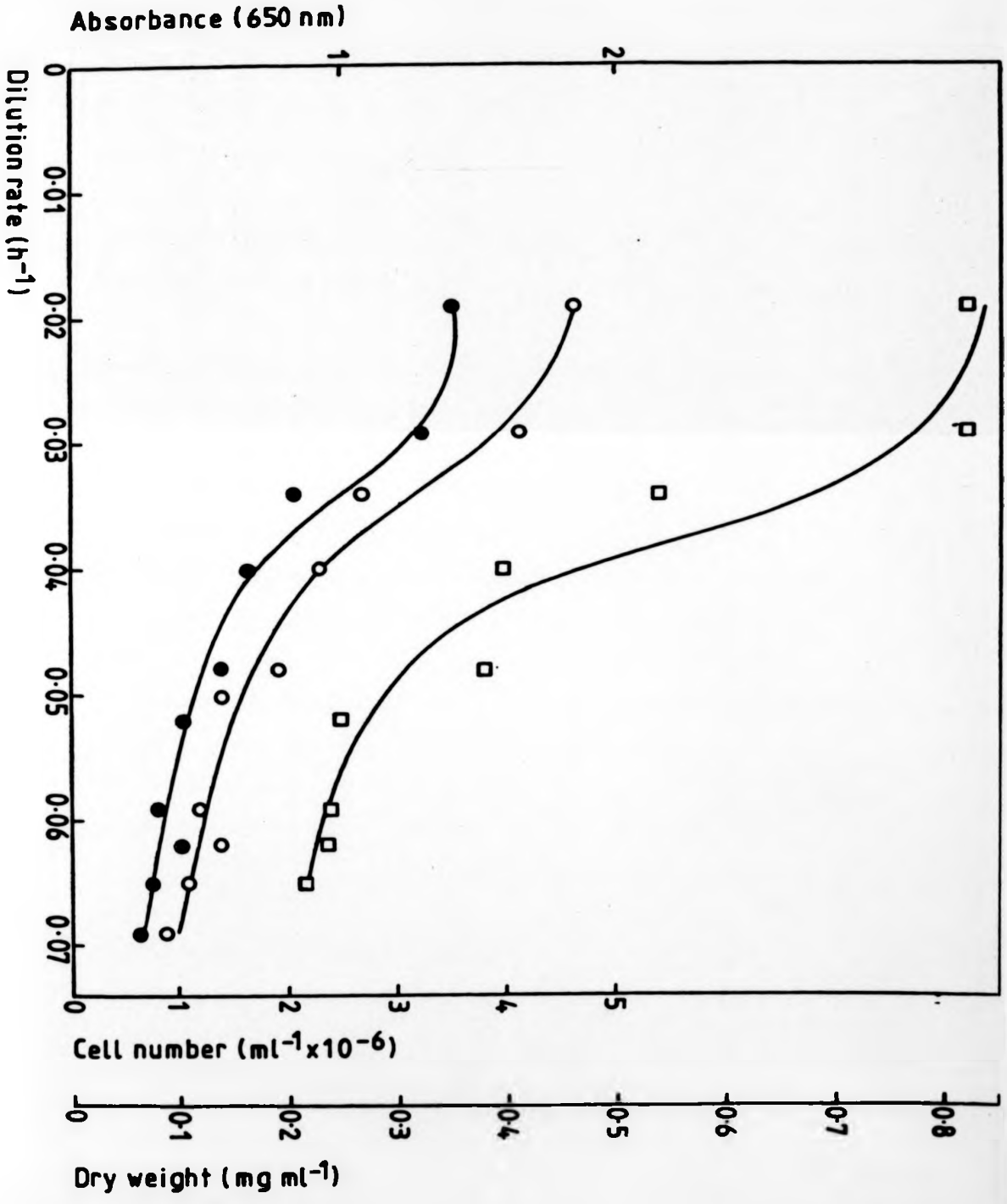


Figure 7.2.

The influence of dilution rate on the steady state biomass concentration, in terms of absorbance (●), cell number ml^{-1} (○) and dry weight (mg ml^{-1}) (□), of S. quadricauda grown in light-limited chemostat culture.


Absorbance (650 nm)
1
2
Dilution rate (h⁻¹)
0
0·01
0·02
0·03
0·04
0·05
0·06
0·07
0
1
2
3
4
5
Cell number (ml⁻¹x10⁻⁶)
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
Dry weight (mg ml⁻¹)

7.2. COMPETITION STUDIES

Competition studies on mixed cultures of *A. nidulans* and *S. quadricauda* were carried out under light- and carbon dioxide-limited chemostat continuous-flow culture conditions at dilution rates of 0.025, 0.035 and 0.045 h^{-1}.

It was found that the initial population sizes of the two organisms was of no importance to the outcome of the competition as similar results were obtained irrespective of the amounts of the initial inocula. For example, at D=0.025 h^{-1} under light-limited conditions in one experiment an inoculum of 1.21×10^6 *S. quadricauda* cells ml^{-1} and 7.8×10^8 *A. nidulans* cells ml^{-1} was used (figure 7.5.) while in a second experiment the numbers of cells ml^{-1} were 0.25×10^6 and 1.6×10^8 respectively. In both cases *A. nidulans* became the competitive, dominant population reaching values of approximately 1×10^9 cells ml^{-1} after 166 hours in the first experiment and after 140 hours in the second. Also, in both cases the numbers of *S. quadricauda* cells decreased rapidly becoming virtually impossible to count accurately after 238 and 214 hours respectively in the first and second experiments. Another example of similar results being achieved when vastly different numbers of the two organisms were used in the initial inoculum was shown at D=0.035 h^{-1} under carbon dioxide-limited conditions (figures 7.6. and 7.7.). As can be seen, in these experiments the results obtained were almost identical with *A. nidulans* rapidly becoming the competitive, dominant population with the numbers of *S. quadricauda* cells decreasing rapidly and

being virtually impossible to count accurately after about 140 hours. Consequently, only one example of the results obtained for each dilution rate under each limitation is given.

Wall growth was found to be a major problem in these experiments as S. quadricauda, in particular, grew readily on the walls of the growth vessel, especially under light-limiting growth conditions. This ability must have conferred a competitive advantage on this organism under these conditions as light was obviously more available close to the walls of the vessel than in the centre of the culture where mutual shading occurred. This phenomenon was shown, for example, at $D=0.035\ h^{-1}$ under light-limiting conditions (figure 7.3.) when the culture vessel was shaken at 550 h after being left for four days. The increased number of S. quadricauda cells present being due to wall growth. Another example was obtained at $D=0.025\ h^{-1}$ under light-limiting conditions. In this case the number of S. quadricauda cells in the culture after 250 h was too low to quantitate accurately but when the culture vessel was shaken and the wall growth removed the number of cells within the culture increased to over 1×10^6 cells ml^{-1}. Therefore, care had to be taken to remove any wall growth that occurred as quickly as possible otherwise accurate results would not have been obtained.

Under certain growth conditions, that is, at very low growth rates and under carbon dioxide-limitation, it was found that A. nidulans became the competitive, dominant population

selectively excluding S. quadricauda (sections 7.2.1. and 7.2.2.). However, despite the fact that the numbers of S. quadricauda cells decreased rapidly until it was impossible to count them accurately, if the conditions were changed to light-limitation at dilution rates of 0.035 or 0.045 h^{-1} then the cell numbers gradually increased. This indicated that coexistence rather than competitive exclusion was occurring in these cultures. Coexistence was also shown to occur under conditions when S. quadricauda was the competitive dominant (section 7.2.).

7.2.1. Light-limited conditions

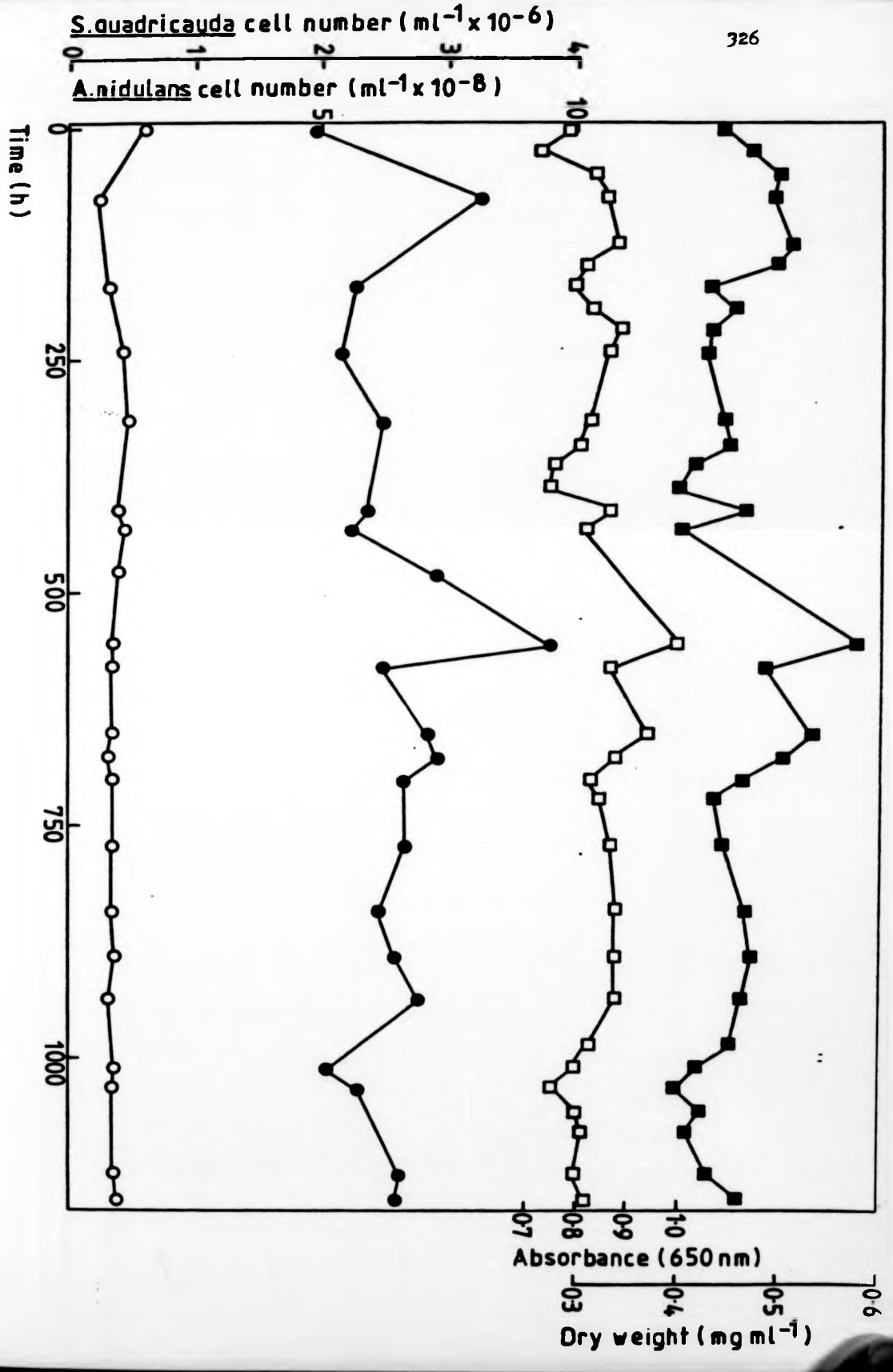
Competition studies on mixed cultures of A. nidulans and S. quadricauda were carried out at dilution rates of 0.025, 0.035 and 0.045 h^{-1}.

At a dilution rate of 0.035 h^{-1}, the numbers of S. quadricauda cells present, approximately 2.4 - 2.8 x 10^6 cells ml^{-1}, were very similar to the number of these organisms present, approximately 3 x 10^6 cells ml^{-1}, under the same conditions in pure culture whereas the numbers of A. nidulans cells present, approximately 1 x 10^8 cells ml^{-1}, were rather lower than those obtained in pure culture, approximately 5 x 10^8 cells ml^{-1} (figure 7.3.). So under these growth conditions S. quadricauda was the competitive, dominant population, although coexistence was obviously occurring.

The actual numbers of the two organisms present was deceptive in these experiments due to the great differences in the cell sizes of the two organisms. If it was assumed that the

Figure 7.3.

Competition study of A. nidulans (○) and S. quadricauda (●), in terms of organisms ml^{-1}, at $D=0.035\ h^{-1}$ in light-limited chemostat culture. The total culture biomass concentration, in terms of absorbance (□) and dry weight (mg ml^{-1}) (■), was continuously monitored.


S.quadricauda cell number (ml⁻¹ x 10⁻⁶)
0
1
2
3
4
A.nidulans cell number (ml⁻¹ x 10⁻⁸)
5
10
Time (h)
0
250
500
750
1000
0·7
0·8
0·9
1·0
Absorbance (650 nm)
0·3
0·4
0·5
0·6
Dry weight (mg ml⁻¹)

cell sizes in these competition studies were approximately the same as obtained for the two organisms in pure culture then, at a dilution rate of 0.035 h^{-1}, the cells of A. nidulans would have been approximately 0.45 pg dry weight $(cell)^{-1}$ and those of S. quadricauda 200 pg dry weight $(cell)^{-1}$. Therefore, in the above experiment the total dry weight of A. nidulans with approximately 1 x 10^8 cells ml^{-1} present would have been approximately 0.045 mg ml^{-1} and that of S. quadricauda with approximately 2.5 x 10^6 cells ml^{-1} present would have been approximately 0.5 mg ml^{-1}. So, despite the fact that there was a greater actual number of A. nidulans cells in this experiment, the majority (about 92%) of the total biomass, in terms of dry weight, was due to S. quadricauda.

At a dilution rate of 0.045 h^{-1}, the numbers of S. quadricauda cells present, approximately 1.6 - 2.0 x 10^6 cells ml^{-1}, were very similar to the number of these organisms present, approximately 1.8 x 10^6 cells ml^{-1}, under the same conditions in pure culture whereas the numbers of A. nidulans cells present, approximately 0.8 x 10^8 cells ml^{-1}, were rather lower than those obtained in pure culture, approximately 2.5 x 10^8 cells ml^{-1} (figure 7.4.). So under these growth conditions S. quadricauda was the competitive, dominant population, although again coexistence was obviously occurring.

At a dilution rate of 0.025 h^{-1}, the numbers of A. nidulans cells increased rapidly to approximately 9 x 10^8 cells ml^{-1} (the same number of these organisms as present under the same conditions in pure culture) whereas the numbers of S. quadricauda

Figure 7.4.

Competition study of A. nidulans (○) and S. quadricauda (●), in terms of organisms ml^{-1}, at D=0.045 h^{-1} in light-limited chemostat culture. The total culture biomass concentration, in terms of absorbance (□) and dry weight (mg ml^{-1}) (■), was continuously monitored.

S.quadricauda cell number (ml⁻¹x10⁻⁶)
0
1
2
3
4
A.nidulans cell number (ml⁻¹x10⁻⁸)
5
10
Time (h)
0
100
200
300
400
0·5
0·6
0·7
0·8
0·9
1·0
Absorbance (650 nm)
0·2
0·3
0·4
0·5
Dry weight (mg ml⁻¹)

cells decreased fairly rapidly and were impossible to count accurately after about 310 hours (figure 7.5.). (Due to the counting method used, the Neubauer counting chamber, cell numbers below about 2×10^4 were impossible to quantify accurately). So under these growth conditions A. nidulans was the competitive, dominant population and S. quadricauda was virtually eliminated from the culture.

7.2.2. Carbon dioxide-limited conditions

Competition studies on mixed cultures of A. nidulans and S. quadricauda were carried out at dilution rates of 0.025, 0.035 and 0.045 h^{-1}.

At a dilution rate of 0.035 h^{-1}, the numbers of A. nidulans cells increased rapidly to approximately 2.8×10^8 cells ml^{-1} whereas the numbers of S. quadricauda cells decreased rapidly and were impossible to count accurately after about 190 hours (figure 7.6.). So under these growth conditions A. nidulans was the dominant organism with S. quadricauda being virtually eliminated from the culture.

However, S. quadricauda had not previously been cultured under carbon dioxide-limiting conditions so it was not known whether competition was actually occurring with A. nidulans for the limiting substrate or whether this organism could not grow under these conditions anyway. Consequently, S. quadricauda was grown in pure culture at a dilution rate of 0.035 h^{-1} under carbon dioxide-limiting conditions and was found to attain a steady state cell number of 1.25×10^6 cells ml^{-1}, so was

Figure 7.5.

Competition study of A. nidulans (○) and S. quadricauda (●), in terms of organisms ml^{-1}, at D=0.025 h^{-1} in light-limited chemostat culture followed by the imposition of carbon dioxide-limiting conditions at 310 h (indicated by arrows).

The total culture biomass concentration, in terms of absorbance (□) and dry weight (mg ml^{-1}) (■), was continuously monitored.

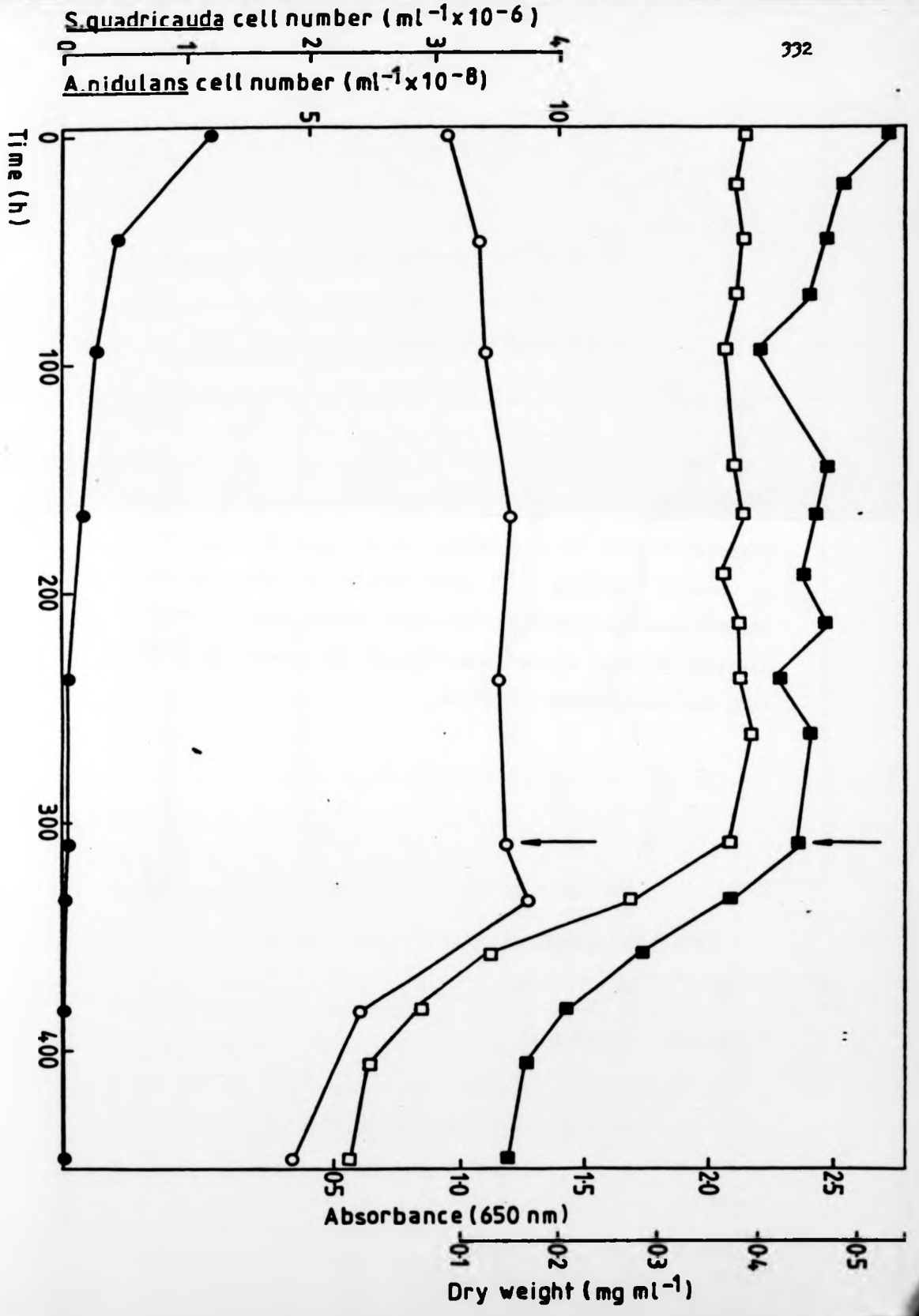
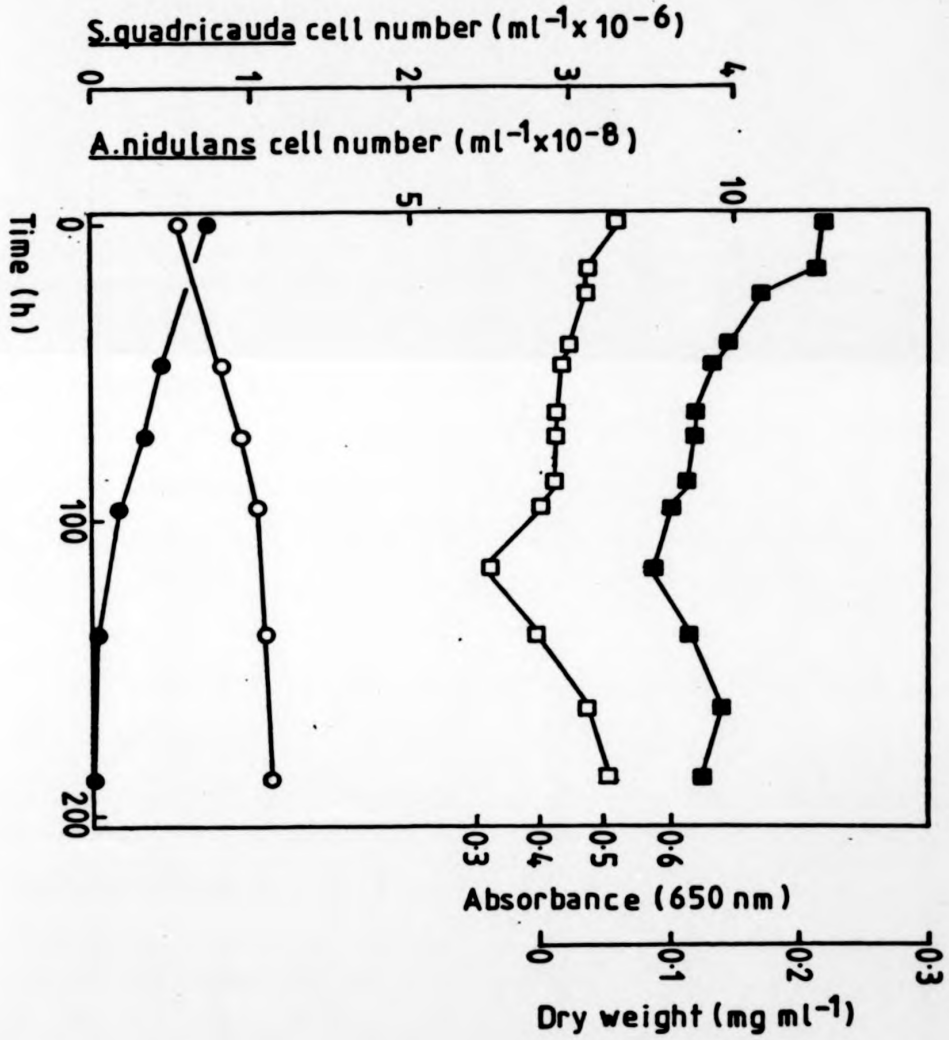

S.quadricauda cell number (ml⁻¹x10⁻⁶)
0
1
2
3
4
A.nidulans cell number (ml⁻¹x10⁻⁸)
5
10
Time (h)
0
100
200
300
400
0·5
1·0
1·5
2·0
2·5
Absorbance (650 nm)
0·1
0·2
0·3
0·4
0·5
Dry weight (mg ml⁻¹)

Figure 7.6.

Competition study of A. nidulans (○) and S. quadricauda (●), in terms of organisms ml^{-1}, at $D=0.035\ h^{-1}$ in carbon dioxide-limited chemostat culture. The total culture biomass concentration, in terms of absorbance (□) and dry weight (mg ml^{-1}) (■), was continuously monitored.


S.quadricauda cell number (ml⁻¹x 10⁻⁶)
0
1
2
3
4
A.nidulans cell number (ml⁻¹x10⁻⁸)
5
10
Time (h)
0
100
200
0·3
0·4
0·5
0·6
Absorbance (650 nm)
0
0·1
0·2
0·3
Dry weight (mg ml⁻¹)

obviously capable of growing under these conditions. An inoculum of A. nidulans cells was added to this steady state S. quadricauda culture at D=0.035 h^{-1} and the competition was followed as before. The results obtained were almost identical to the previous experiment (figure 7.6.) with the numbers of A. nidulans cells increasing rapidly and the numbers of S. quadricauda cells decreasing rapidly (figure 7.7) so indicating that A. nidulans was the competitive, dominant population.

At a dilution rate of 0.045 h^{-1}, the numbers of A. nidulans cells increased rapidly to approximately 2 x 10^8 cells ml^{-1} whereas the numbers of S. quadricauda cells decreased rapidly and were impossible to count accurately after about 140 hours (figure 7.8.). So under these growth conditions A. nidulans was again the competitive, dominant population with S. quadricauda being virtually eliminated from the culture.

The carbon dioxide-limited competition experiment at a dilution rate of 0.025 h^{-1} was, in fact, carried on from the corresponding light-limited experiment (figure 7.5.) by altering the medium inflow after 310 hours to include the relevant bicarbonate concentration and sparging with air only rather than the CO_2/air mixture. The numbers of A. nidulans cells decreased as expected due to the change to carbon dioxide-limiting conditions although this organism was still the competitive, dominant population and the numbers of S. quadricauda cells continued to decrease with this organism being very rapidly virtually eliminated from the culture (figure 7.5.).

Figure 7.7.

Competition study of A. nidulans (O) and S. quadricauda (●), in terms of organisms ml^{-1}, at $D=0.035\ h^{-1}$ in carbon dioxide-limited chemostat culture. (In this experiment the inoculum of A. nidulans was added to a steady state S. quadricauda culture at $D=0.035\ h^{-1}$). The total culture biomass concentration, in terms of absorbance (□) and dry weight (mg ml^{-1}) (■), was continuously monitored.

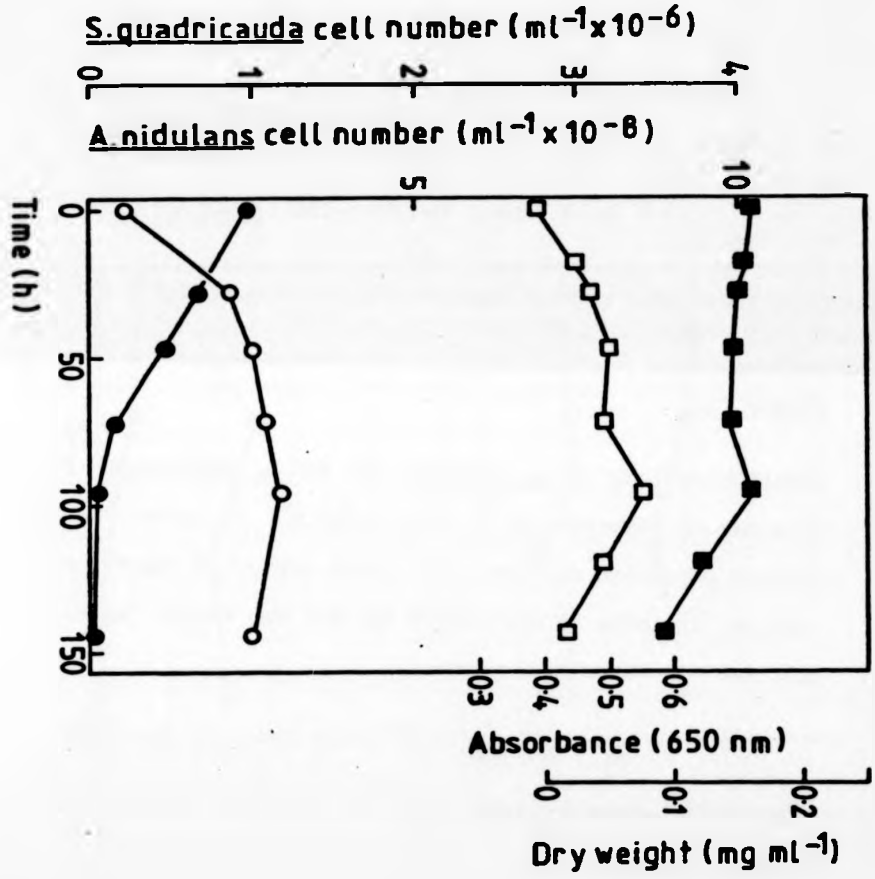
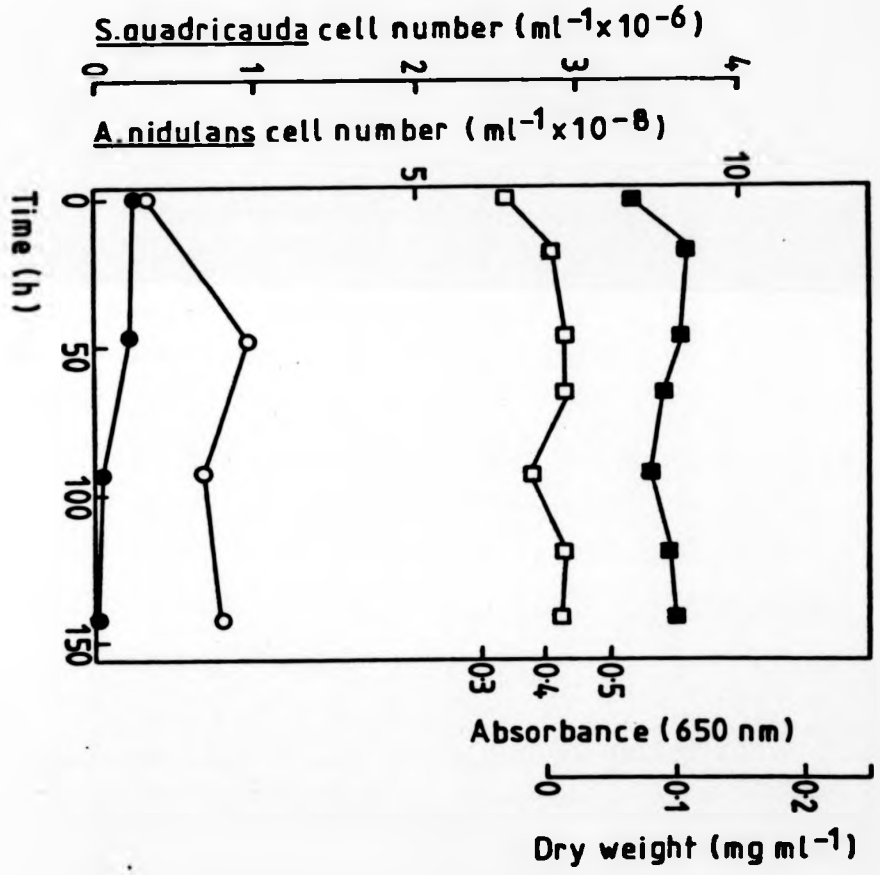

S.quadricauda cell number (ml⁻¹x10⁻⁶)
0
1
2
3
4
A.nidulans cell number (ml⁻¹x10⁻⁶)
5
10
Time (h)
0
50
100
150
0·3
0·4
0·5
0·6
Absorbance (650 nm)
0
0·1
0·2
Dry weight (mg ml⁻¹)

Figure 7.8.

Competition study of A. nidulans (O) and S. quadricauda (●), in terms of organisms ml^{-1}, at D=0.045 h^{-1} in carbon dioxide-limited chemostat culture. The total culture biomass concentration, in terms of absorbance (□) and dry weight (mg ml^{-1}) (■), was continuously monitored.


S.quadricauda cell number (ml⁻¹x10⁻⁶)
0
1
2
3
4
A.nidulans cell number (ml⁻¹x10⁻⁶)
5
10
Time (h)
0
50
100
150
0·3
0·4
0·5
Absorbance (650 nm)
0
0·1
0·2
Dry weight (mg ml⁻¹)

7.3. DISCUSSION

In preliminary experiments carried out to determine the optimal growth medium for use in the competition studies it was found that A. nidulans grew very poorly in GAM with a very low μ_{max} (section 2.10.1.) and an extremely short growth period before a rapid decline. This indicated that this organism could not utilise urea as a nitrogen source, as the nitrogen source was the only major difference between GAM which contained urea and Medium C which contained nitrate-nitrogen. This result was in agreement with observations made by Kratz and Myers (1955) and Birdsey and Lynch (1962) that A. nidulans could not utilise urea as a nitrogen source. There have, however, been reports that other cyanobacteria could utilise urea (Kratz and Myers, 1955; Berns, Holohan and Scott, 1966; Fogg, Stewart, Fay and Walsby, 1973). On the other hand, S. quadricauda grew readily on both mineral media indicating that this organism could utilise both urea and nitrate as nitrogen sources. This was in agreement with the results of several workers who found that members of the Chlorophyceae, including several Scenedesmus species, contained ATP-urea amidolyase (UALase) which catalysed the ATP-dependent degradation of urea (Birdsey and Lynch, 1962; Leftley and Syrett, 1973; Bekheet and Syrett, 1977). It was also found that algal groups other than the Chlorophyceae contained urease rather than UALase as do bacteria, fungi and higher plants.

The basic growth characteristics shown by both A. nidulans and S. quadricauda in pure culture with changing dilution rate

under light-limited continuous-flow culture conditions were similar to those found previously for A. nidulans under light-limited conditions (section 3.3.). This indicated that the rapid decrease in organism biomass with increasing growth rate was a general phenomenon under light-limited conditions irrespective of the organism or other growth conditions of, for example, temperature and was in agreement with the results of other workers (section 1.4.5.). Although the basic growth characteristics were similar, A. nidulans was unable to achieve steady state growth rates above 0.05 h^{-1} under these growth conditions. This was presumed to be due to the decreased temperature and aerobic growth conditions. The growth temperature, about 27°C, was significantly lower than the optimum growth temperature for this organism of 40°C and it has been previously shown by Karagouni (1979) that temperatures below this optimal value adversely affect the growth of this organism. Also, according to Fogg et al. (1973) cyanobacterial growth is favoured by low levels of oxygen with these organisms tending to grow more rapidly under microaerophilic than fully aerobic conditions.

The competition studies showed that under carbon dioxide-limitation at all the dilution rates examined A. nidulans was the competitive, dominant population whereas under light-limitation A. nidulans was dominant at D=0.025 h^{-1} and S. quadricauda at dilution rates of 0.035 and 0.045 h^{-1}. These results indicated that under carbon dioxide-limiting growth conditions the saturation curves for these two organisms for this substrate

did not overlap and as A. nidulans displaced S. quadricauda at each growth rate examined presumably this organism had a lower saturation constant for carbon dioxide (supplied in the form of bicarbonate). This would be consistent with the conclusions of Shapiro (1973) who found that cyanobacteria seemed to be more efficient at obtaining carbon dioxide from low concentrations than green algae. The greater efficiency for the fixation of carbon dioxide may have been due to the possible ability of A. nidulans to fix carbon dioxide by a C_4 pathway, especially at low dilution rates, due to the activity of PEPCase under carbon dioxide-limited conditions (section 6.2.2.). On the other hand, green algae, such as Chlorella, for example, are known to fix carbon dioxide solely by the Calvin cycle (Colman, Cheng and Ingle, 1976) which is less efficient than the C_4 pathway.

The results for the competition studies under light-limited growth conditions indicated that the saturation curves for the two organisms crossed between dilution rates of 0.025 and 0.035 h^{-1} with A. nidulans being dominant at the low growth rate and S. quadricauda becoming dominant at higher growth rates. S. quadricauda would have been expected to be the better competitor at the higher growth rates due to the higher maximum specific growth rate value obtained for this organism under these conditions. These results were in agreement with those obtained by Mur, Gons and van Liere (1977) who found that the cyanobacterium Oscillatoria agardhii was dominant at low light intensities and

dilution rates up to 0.03 h^{-1} whereas the green alga Scenedesmus protuberans was dominant at high light intensities. It was concluded that cyanobacterial growth is generally favoured by low light intensities. This would explain the dominance of A. nidulans at the low dilution rate of 0.025 h^{-1} when due to the high population density under these conditions mutual shading would cause a diminishing average light intensity within the culture vessel. At higher growth rates, however, shading effects would presumably be reduced due to the lower population densities so allowing S. quadricauda to become dominant. This was also in agreement with the results of Shapiro (1973) who found that high concentrations of carbon dioxide and nutrients, as were present in these experiments, led to dominance by green algae.

Despite the fact that one organism in these experiments was always competitively dominant over the other, coexistence was always found to occur to a certain extent. This was most noticeable in the experiments when S. quadricauda was dominant, although the green alga was obviously still present in low numbers when A. nidulans was dominant. A similar situation was shown by Meers (1971) who found that Bacillus subtilis was competitively dominant over Torula utilis at a dilution rate of 0.08 h^{-1}. However, if the culture was left until T. utilis was undetectable and then the dilution rate decreased to 0.05 h^{-1}, the yeast was found to gradually increase in numbers indicating that coexistence was occurring. Coexistence has, according to Slater and Godwin (1980), been shown in a number of competition and selection experiments

in which the uncompetitive populations were always retained at low levels ranging from 0.001 - 1% of the total population. It was concluded that although growth system artifacts could not be completely excluded, the possibility did exist that some mechanism as yet undefined was operating to retain unwanted genes within the population as a whole despite any resulting growth rate disadvantage.

The mixed populations obtained in these experiments reached a seemingly stable situation very rapidly and maintained their respective population densities for long periods of time. The experiment at $D=0.035\ h^{-1}$ under light-limiting conditions was, for example, run for 1150 hours (figure 7.3.). This phenomenon of coexistence on a single-limiting substrate was not consistent with the theory of competition between two species under such conditions which states that such competition should result in the selective exclusion of all but one species (section 1.5.). A corresponding example of coexistence was obtained for two species of Thiobacillus under thiosulphate-limited conditions (Smith and Kelly, 1979) and it was concluded that interactions other than that of competition for the limiting substrate might be occurring. In the present study, however, the relative initial population sizes of the two organisms was found to be of no importance indicating, according to Jannasch and Mateles (1974), that competition was the sole interaction between these two species. This fact also indicates that neither of these two organisms produced extracellular metabolites which had a significant

effect on the other as this effect would have been dependent on initial inocula concentrations (Meers and Tempest, 1968; Lam and Silvester, 1979). This independence of initial population density was shown particularly in the case of A. nidulans when a small inoculum was added to the steady state culture of S. quadricauda at D=0.035 h^{-1} under carbon dioxide-limiting conditions (figure 7.7.) and A. nidulans assumed dominance as rapidly as in a previous experiment when the two organisms had been added together at more equal biomass concentrations (figure 7.6.). These results, and the observations of Slater and Godwin (1980), therefore, tend to indicate that coexistence might be a more general phenomenon than was previously thought with complete exclusion of all but one species due to competition for a single limiting substrate possibly being rare. On the other hand more complex interactions could be occurring between the two species in these experiments although the nature of these interactions are unknown.

It is obviously impossible to correlate the results obtained from these experiments directly with natural systems but a number of situations were indicated in which the growth of cyanobacteria would be expected to be favoured over that of eukaryotic algae, such as at low light intensities, high water temperatures, low oxygen concentrations and under carbon dioxide-limiting conditions. All of these conditions have, in fact, been shown to be important in the succession of cyanobacteria in natural situations (section 1.5.).

After these experiments had been completed, it was attempted to emulate the natural situation more closely by decreasing the culture temperature. However, this was not at all successful as A. nidulans did not grow well at temperatures below 25°C - the culture becoming yellow in colour, presumably due to the loss of pigments, and the cells tending to clump together. This was presumably a problem of using a 'laboratory' culture for these experiments which had been adapted for many years to grow at a temperature of 40°C. It would have been interesting to obtain a 'natural' cyanobacterium and carry out similar experiments to see if this would behave in a similar manner to the laboratory organism. Further work could also have been carried out to determine the effects of nutrient limitation, such as phosphate and nitrate, on the outcome of competition between a cyanobacterium and a green alga to see if the effects of these factors could be correlated with the situation known to occur under natural conditions. Such experiments could be important in increasing knowledge of the causes of cyanobacterial bloom formation.

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APPENDIX

PARROTT, L.M. and SLATER, J.H. (1979). The effect of growth rate on the macromolecular composition of Anacystis nidulans grown in light-and carbon dioxide-limited chemostat cultures. Proceedings of the Society for General Microbiology, 6, 76.

G15 *The Effect of Growth Rate on the Macromolecular Composition of Anacystis nidulans Grown in Light- and Carbon Dioxide-Limited Chemostat Cultures.* Linda M. Parrott and J.H. Slater (Department of Environmental Sciences, University of Warwick, Coventry CV4 7AL).

The basic macromolecular composition, DNA, RNA, protein and pigment content, of *Anacystis nidulans* was determined in light- and carbon dioxide-limited chemostat cultures over the dilution rate range, D = 0.02 to 0.20 h^{-1}. Under both limitations the RNA content per cell increased with increasing D over the above range (20 to 55 fg $cell^{-1}$). On a % of dry weight basis the RNA content increased with increasing specific growth rate although the values were higher for carbon dioxide-limited organisms (4 to 8%) compared with light-limited (4.5 to 5.5%). The specific rate of RNA synthesis, q_{RNA}, increased with increasing D for both limitations. The DNA content as a % of dry weight decreased approximately two fold with increasing D. However, for both limitations, the DNA content per cell reached a maximum at D = 0.1h^{-1} (4.5 fg $cell^{-1}$ for light-limited organisms and 8.0 fg $cell^{-1}$ for carbon dioxide-limited organisms) but then declined with increasing D up to 0.2 h^{-1}. The q_{DNA} increased up to D = 0.1 h^{-1} but, unlike q_{RNA}, at high dilution rates q_{DNA} appeared to be unrelated to the growth rate. Under light-limited conditions the protein content per cell and as a % dry weight showed a similar pattern to the DNA curves. Under carbon dioxide-limited conditions the protein content per cell increased over the D range examined.

The pigment analyses on chlorophyll *a*, carotenoid and phycocyanin content showed that phycocyanin was the main contributor to the total pigment content. In general on a per cell basis, the amount of each pigment increased with increasing growth rate although the actual amounts were higher under carbon dioxide-limitation than under light-limitation. On a dry weight basis there was a similar increase in pigment content under carbon dioxide-limitation but under light-limitation the pigment content decreased above a dilution rate of approximately 0.07 h^{-1}.